Supporting Information

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SI Text

Alkaline Phosphatase Assay. Assays were performed, and units of activity were calculated as described (1). Strain CC191pJDT3 lacking the FimH SP was used to subtract background signal (average of 2.3% of the signal of LS4–4-20, ± SEM of 1.1). Signal from strains LS4–4-1, LS4–4-27, LS3–31-2, and LS4–4-8 was normalized to signal from LS4–4-20. The average of this ratio was calculated for four independent experiments.

Flow Cytometry. A total of 10^8 cells were resuspended in PBS with 0.2% BSA (PBS-BSA), 1% α-methyl-D-mannopyranoside (ammp; Sigma), and Fab fragments of mAb21 (recognizes FimH) and incubated for 2 h on ice. Cells were washed twice and resuspended with flow cytometry grade, anti-mouse IgG (Fab specific), FITC-conjugated F(ab′)2 from goat (Sigma) at a dilution of 1:100 in PBS-BSA. After incubating for 1.5 h on ice, samples were washed twice and resuspended in PBS at 10^8 cells/ml. Fluorescence of 100,000 particles per sample was measured on a FACScan (Becton Dickinson). FlowJo was used for data analysis. For each experiment, the same circular gate based on forward and side scatter (aimed at including only single cells) was applied to all samples. The median fluorescence intensity for each sample was calculated. LSpBB lacking FimH was used to subtract background signal. Signal from strains LS60 (T-6N), LS68 (T-6N/V-10I), LS154 (T-6N/V-10A), and LS141 (V-4E) was normalized to signal from LS76 (WT). The average of this ratio was calculated for five independent experiments.

Adhesion to T24 Uroepithelial Cells. Strains LS76, LS60, LS68, LS154, LS141, and LSpBB were tested as described (2). T24 cells (HTB-4), derived from a human bladder carcinoma cell line and obtained from ATCC, were grown to confluence in 24-well tissue culture plates (greiner bio-one) at 37°C under 5% CO_2 and in McCoy's 5A medium (GIBCO) supplemented with 10% FBS, penicillin, and streptomycin. Before the assay, T24 cells were washed with HBSS (GIBCO). Bacterial strains were resuspended in MaCoy's 5A medium to 10^7 cells/ml with or without 3% ammp. After incubation of bacteria and T24 cells for 2 h, wells were washed six times with HBSS and treated with Triton X-100, and contents were diluted and plated to quantify the number of adherent bacteria. Experiments were performed in quadruplicate and repeated three times. Wells lacking T24 cells did not result in any cfus.

Isolation of Human Neutrophils. According to a protocol approved by the University of Washington Human Subjects Division, human neutrophils were isolated from venous blood of volunteers under informed consent as described (3). For all preparations, >95% of cells were viable. Flow cytometry showed <1% contaminating peripheral blood mononuclear cells and lymphocytes. Neutrophils were chilled on ice at the end of the procedure for synchronization and used immediately at a concentration of 10^6 cells/ml in RMPI medium 1640 (GIBCO) with 10 mM Hepes (Sigma) (RMPI/H).

Neutrophil Bactericidal and Adherence Assays. Strains LS76, LS60, LS68, LS154, LS141, and LSpBB were adjusted to 10^6 cells/ml in RMPI/H and mixed with an equal volume of neutrophils. Killing and binding were measured as described (4) without plasma or antibodies and a 2.3-h incubation time for killing. When used, ammp was at 3%. Within each experiment, the fraction of bacteria killed (1 − concentration of bacteria incubated with/without neutrophils) was divided by the WT fraction killed (0.72 ± 0.03, SEM). Bacterial growth rates during incubation were similar. For binding, a 30-min, 4°C incubation with inversion every 10 min was used before differential centrifugation. Control samples lacking neutrophils showed removal of unbound bacteria. Samples were assayed in duplicate and the experiments were repeated four times.

1. Michaelis S, Inouye H, Oliver D, Beckwith J (1983) Mutations that alter the signal sequence of alkaline phosphatase in *Escherichia coli*. *J Bacteriol* 154:366–374.
2. Zunino P, *et al*. (2003) Proteus mirabilis fimbriae (PMF) are important for both bladder and kidney colonization in mice. *Microbiology* 149:3231–3237.
3. Voyich JM, DeLeo FR (2002) Host–pathogen interactions: Leukocyte phagocytosis and associated sequelae. *Methods Cell Sci* 24:79–90.
4. Nazareth H, Genagon SA, Russo TA (2007) Extraintestinal pathogenic *Escherichia coli* survives within neutrophils. *Infect Immun* 75:2776–2785.

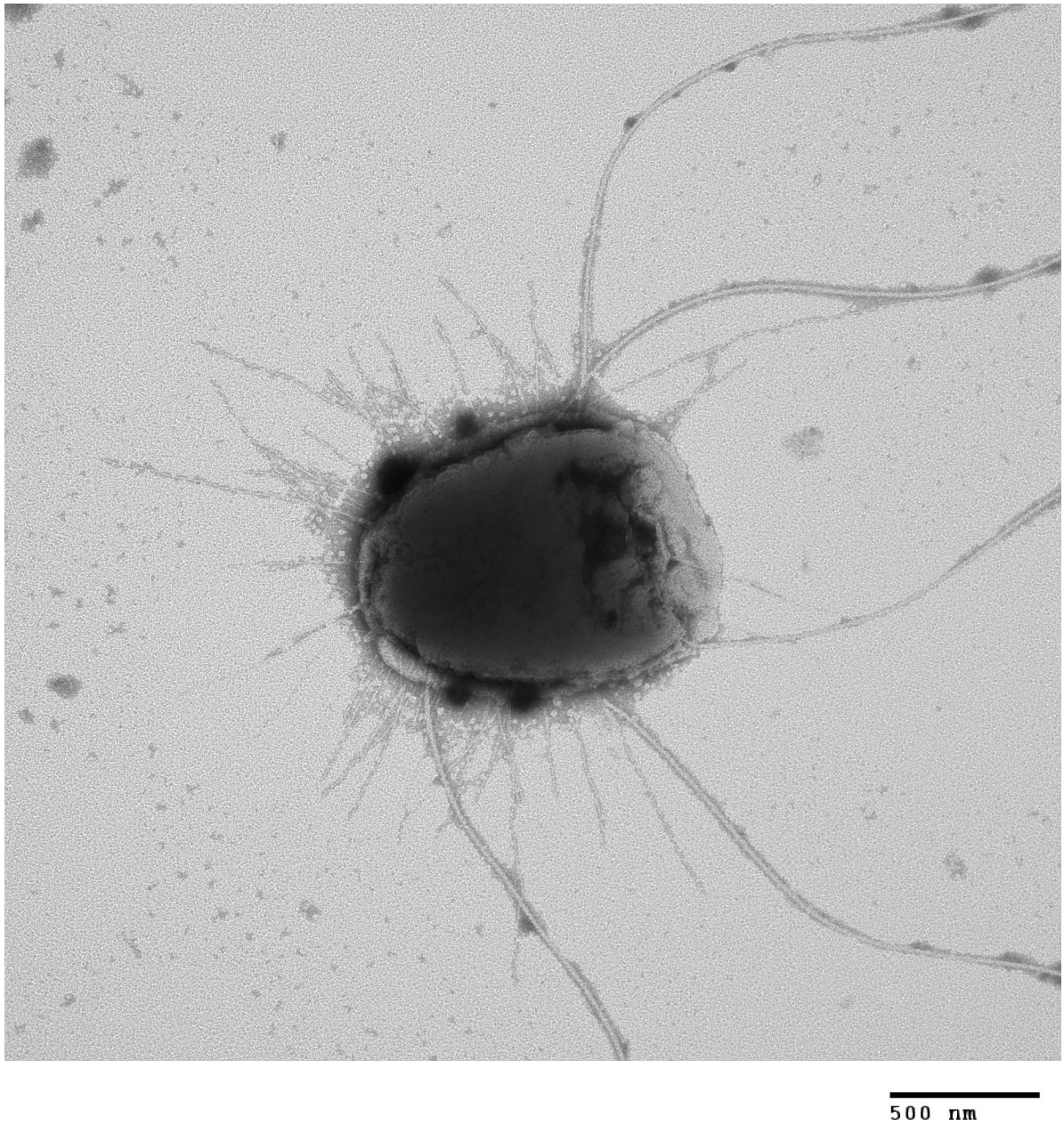

Fig. S1. Electron micrograph of strain LS76 (WT FimH SP)

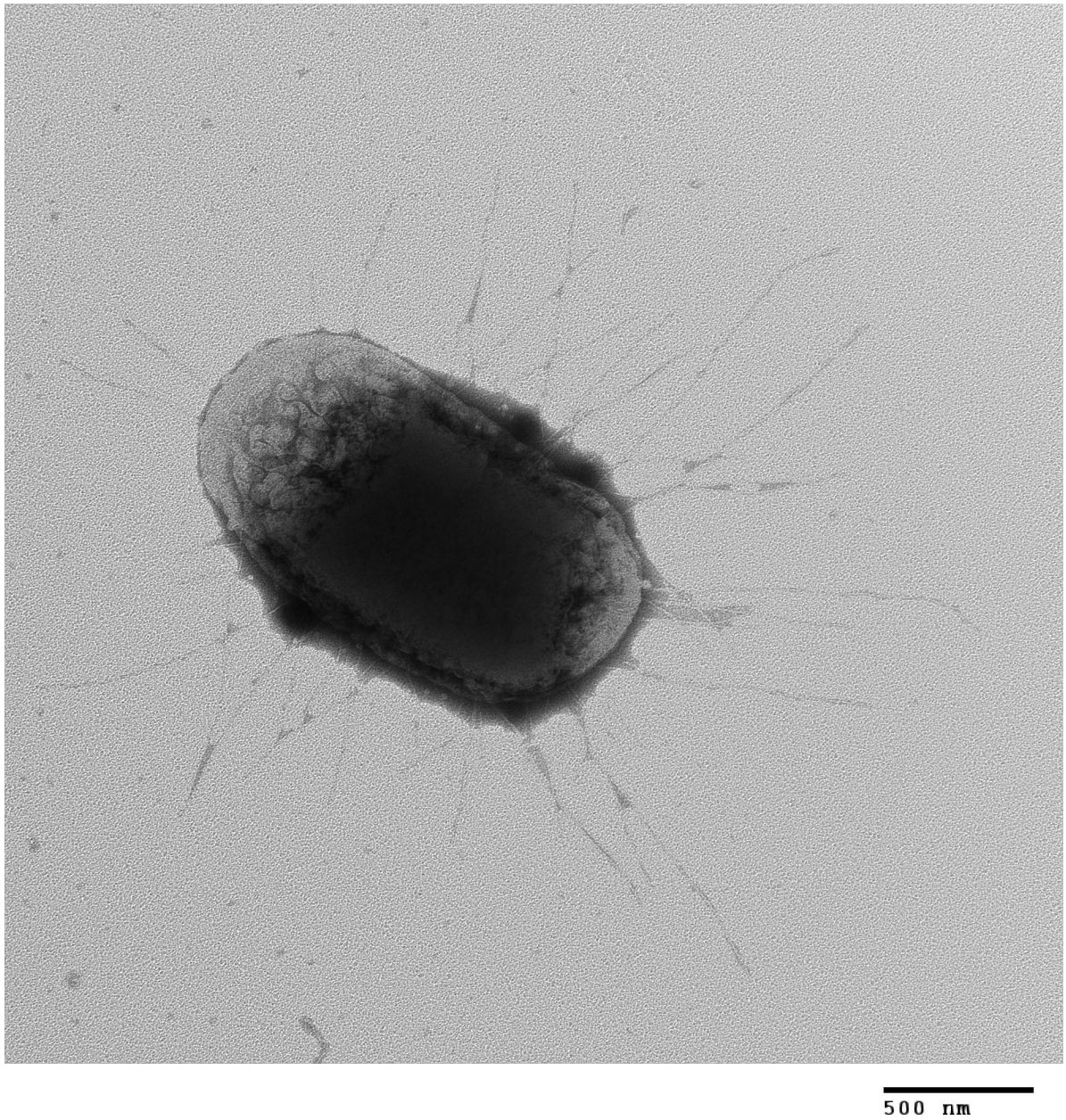

Fig. S2. Electron micrograph of strain LS60 (T-6N FimH SP)

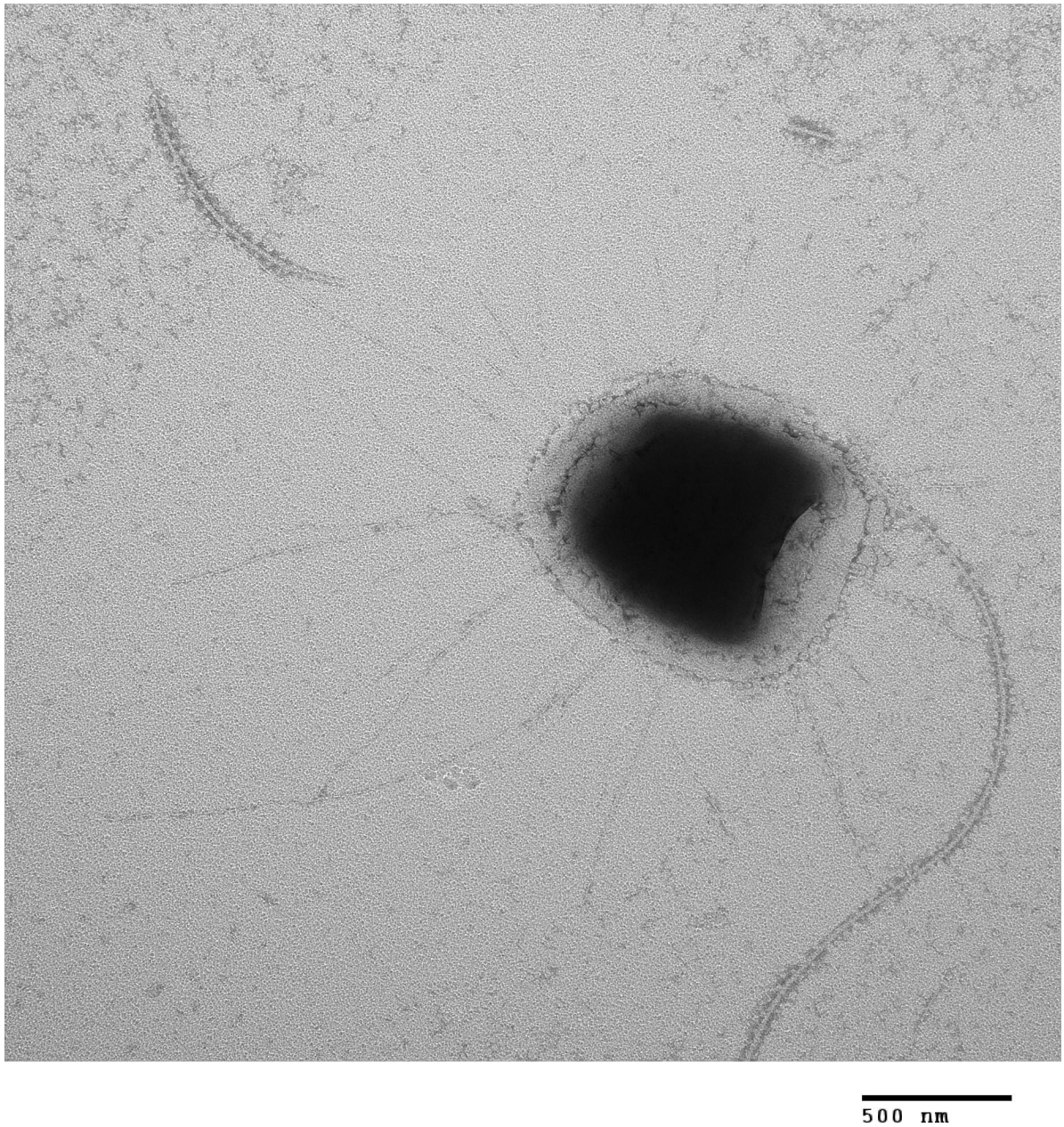

Fig. S3. Electron micrograph of strain LS68 (T-6N/V-10I FimH SP)

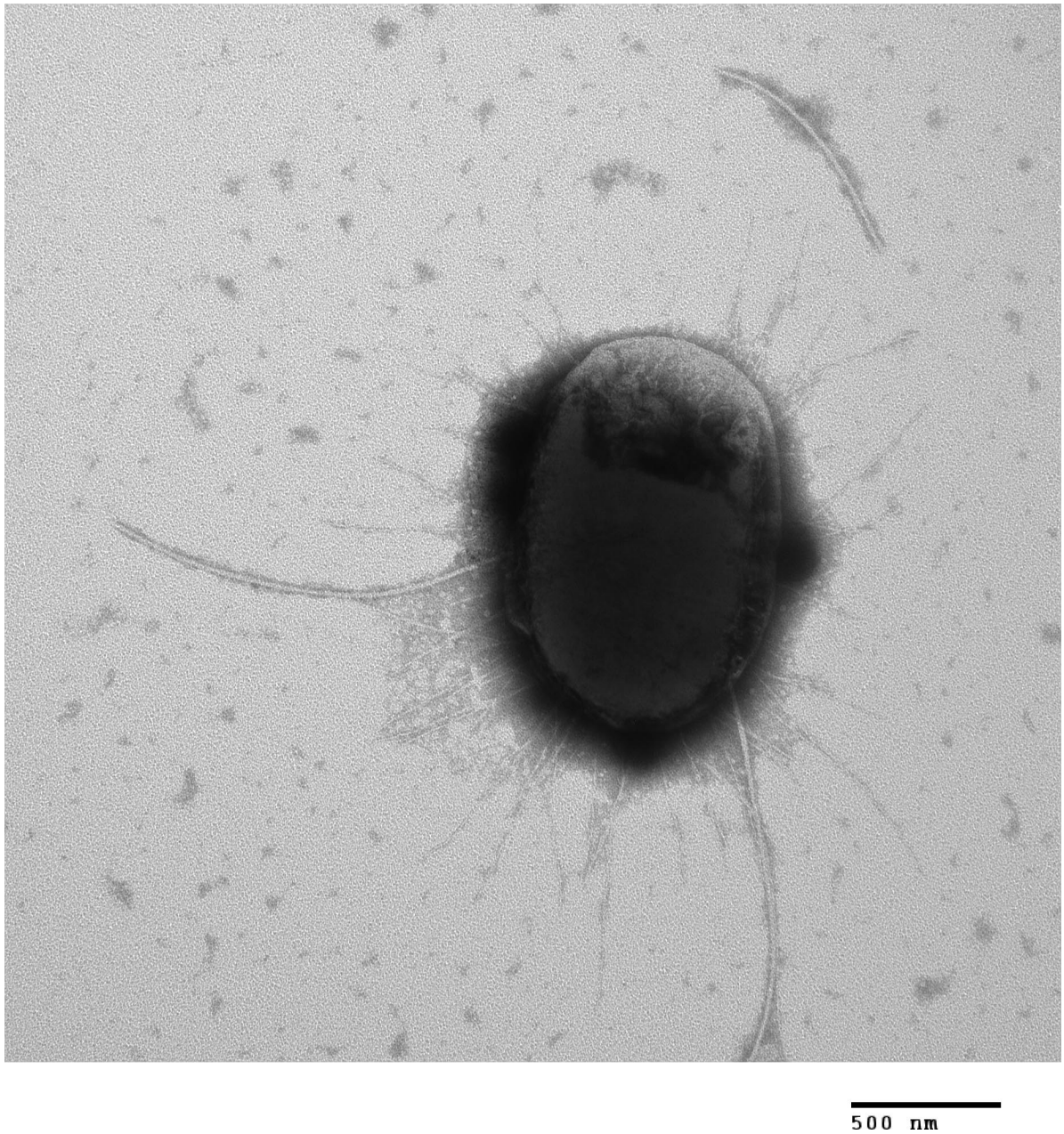

Fig. S4. Electron micrograph of strain LS154 (T-6N/V-10A FimH SP)

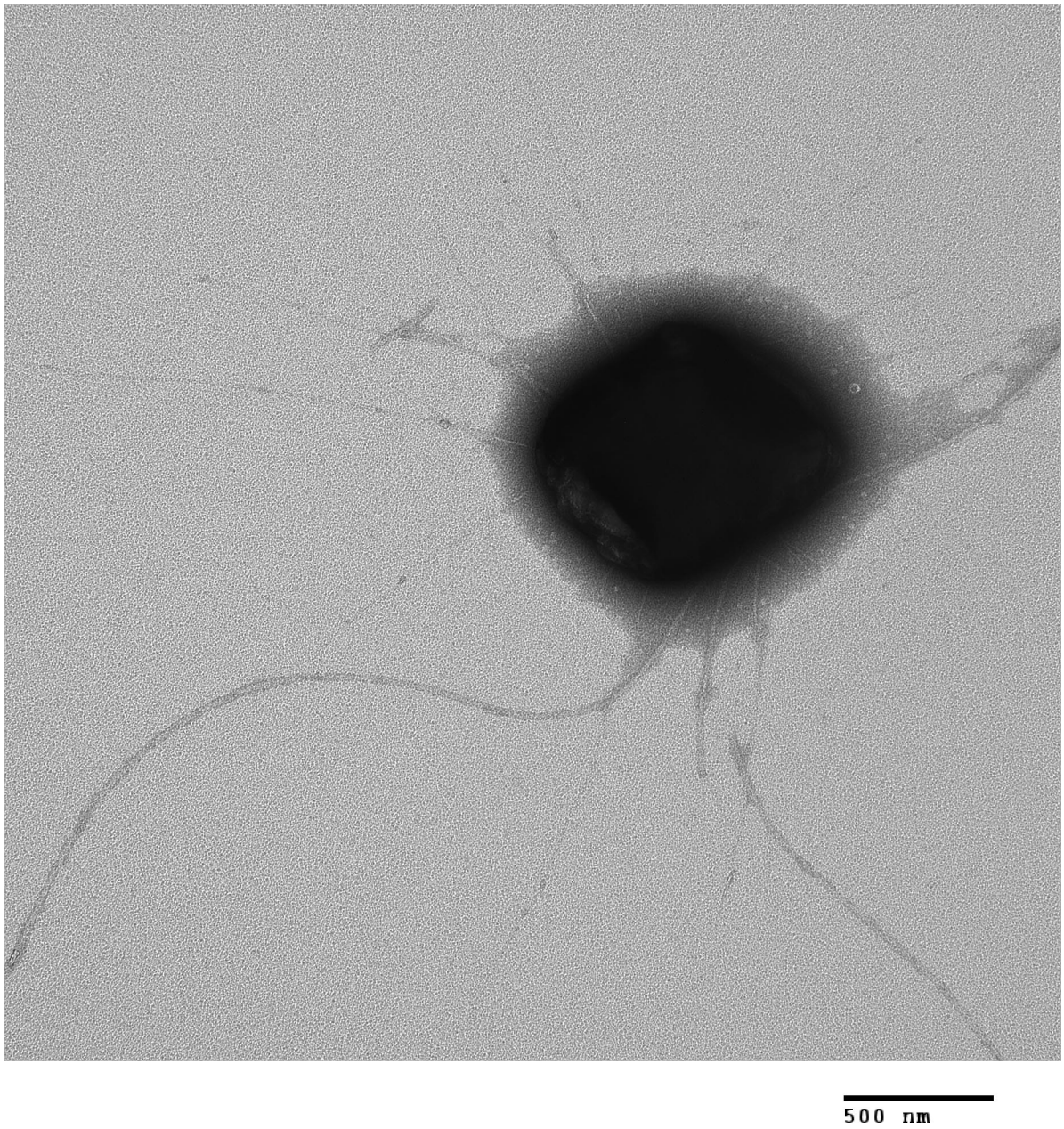

Fig. S5. Electron micrograph of strain LS141 (V-4E FimH SP)

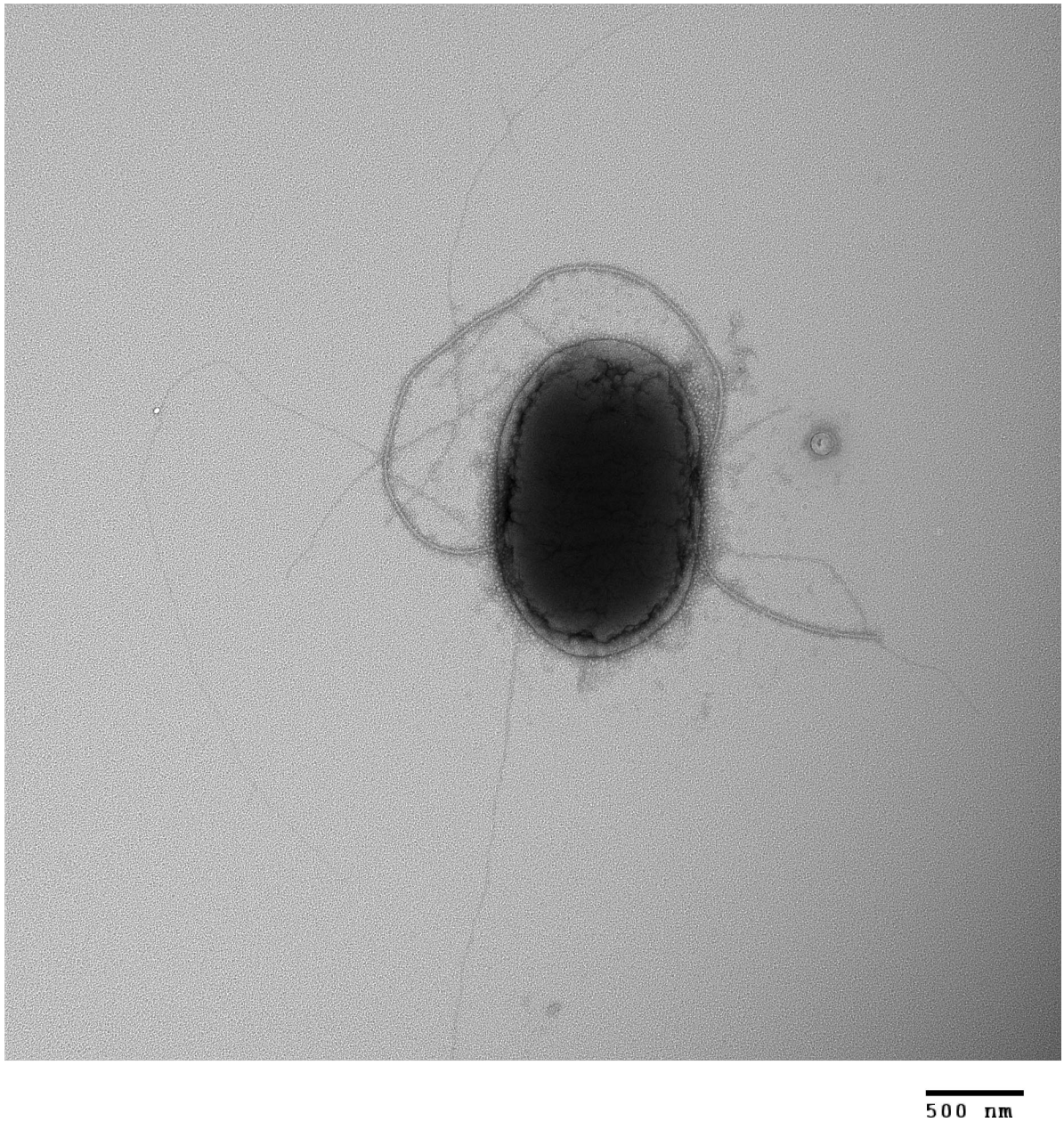

Fig. S6. Electron micrograph of strain LSpBB (no FimH)

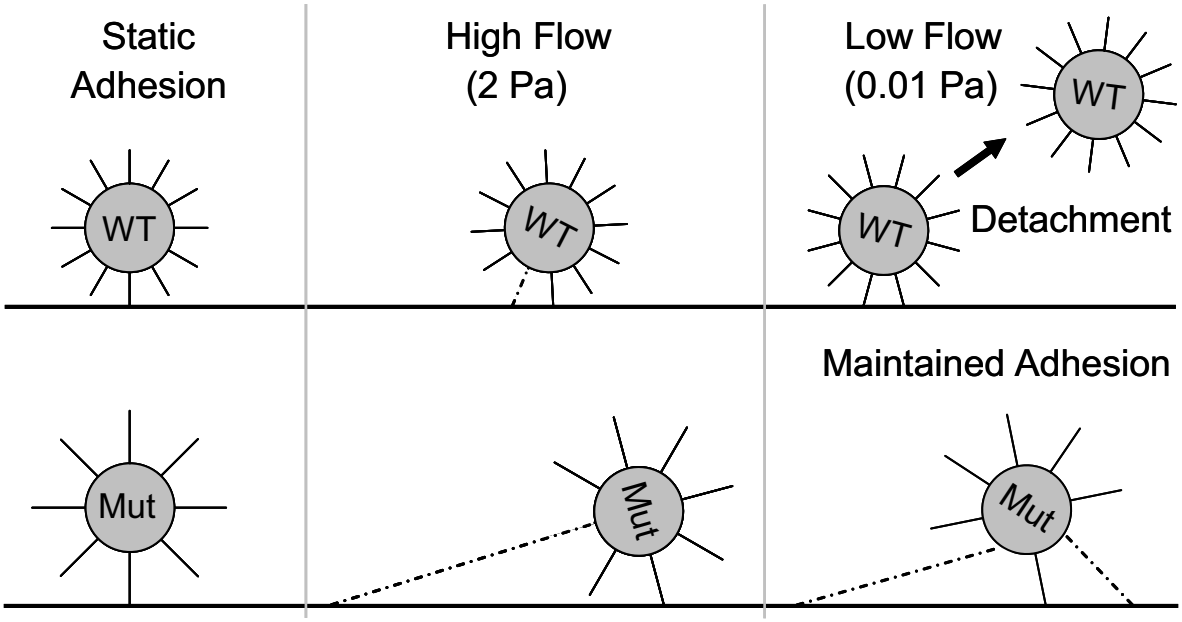

Fig. S7. Potential mechanism by which fewer and longer fimbriae maintain binding after a switch from high to low flow. In the WT, which has many short fimbriae, one fimbria binds and uncoils only a short distance before a second binds. At 2 Pa there is not enough force to uncoil a second fimbria and the bacterium stops. When the flow is decreased to 0.01 Pa, the bacterium rocks back a short distance and the fimbriae completely recoil, and with decreased force on the FimH bonds the bacterium detaches. In contrast, in the mutant (Mut), the longer fimbriae allow for greater uncoiling, and the smaller number of fimbriae means movement is less likely to be stopped by another bond forming. The attached fimbria can uncoil to a length much longer than the bacterium itself. When the flow is decreased, the bacterium is moved back until it is suspended between partially uncoiled fimbriae. The two fimbriae pull on each other's catch bonds, keeping them both in a high-affinity state.

Table S1. Correlation in wild strains between mutations in the SP of FimH and other fimbrial proteins and predicted functional effects

	position and nature of variation				predicted functional effect	FimH			
						WT	Mutants		
	-	-	-	-		- -	- -	- -	- -
	0	1	2	2		0 1	0 1	0 1	0 1
	9	9	0	1		6 0	6 0	6 0	6 0
	V	**T**	**A**	**A**	**-**	**T V**	N V	N I	N A
						-	_	_	-
FimA	V	A	A	A	↓	191	32	3	1
	V	T	V	A	↑	124	2	0	0
	I	T	V	A	↑	6	0	0	0
	V	T	G	A	↓	3	0	0	0
	V	T	A	S	↓	2	0	0	0
	-								
	0								
	4								
FimI	**K**				**-**	153	22	1	1
	R				-	0	1	0	0
	N				↓	0	0	1	0
	-	-	-	-					
	0	1	2	2					
	8	5	4	9					
FimC	**V**	**T**	**M**	**M**	**-**	134	22	2	1
	V	T	M	V	↓	47	0	0	0
	V	T	I	M	↓	7	0	0	0
	L	T	M	M	-	1	0	0	0
	V	I	M	M	-	1	0	0	0
	-	-	-						
	0	2	3						
	8	5	1						
FimD	**L**	**G**	**F**		**-**	12	4	1	0
	L	G	V		↓	2	0	0	0
	I	V	F		↓	1	0	0	0
	-	-							
	1	2							
	9	0							
FimF	**H**	**A**			**-**	53	21	2	1
	R	V			↓	10	0	0	0
	-	-	-	-					
	0	0	1	1					
	4	9	3	6					
FimG	**R**	**L**	**I**	**L**	**-**	31	0	0	0
	C	V	I	L	↓	19	21	2	1
	R	L	M	F	↑	13	0	0	0
	C	L	M	F	↑	7	0	0	0
	R	V	M	F	↑	5	0	0	0
	Y	V	I	L	↓	2	0	0	0
	C	L	M	L	-	0	1	0	0

For each protein of the *fim* cluster, the polymorphic positions of the SP and the amino acid at these sites are indicated. The phylogenetically primary (WT) haplotype is listed first in bold and is given a baseline level of function (−). An improvement (up arrow), decrease (down arrow), or no change (−) in function relative to WT is indicated for each mutant. This change in SP function was experimentally determined for FimH and computationally predicted based on amino acid properties and SignalP software for the remaining proteins. Strains from each of the four phylogenetically independent FimH SP lineages (as reported in Fig. 1*A*) are included.

Table S2. *E. coli* strains and plasmids used in this study

Strain	Relevant chromosomal genotype	Parent strain	Plasmid (FimH SP allele)	Source
CC191	Δ*phoA*	K-12	None	1
CC191pJDT3	Δ*phoA*	CC191	pJDT3-no FimH SP	This study
LS4–4–20	Δ*phoA*	CC191	pJDT3(WT)	This study
LS4–4–1	Δ*phoA*	CC191	pJDT3(T-6N)	This study
LS4–4–27	Δ*phoA*	CC191	pJDT3(T-6N/V-10I)	This study
LS3–31–2	Δ*phoA*	CC191	pJDT3(T-6N/V-10A)	This study
LS4–4–8	Δ*phoA*	CC191	pJDT3(V-4E)	This study
CI10–9	Δ*fimH*	CI10 (wild)	None	2
LSpBB	Δ*fimH*	CI10–9	pPKL9, pBeloBAC11-no FimH	This study
LS76	Δ*fimH*	CI10–9	pPKL9, pBeloBAC11(WT)	This study
LS60	Δ*fimH*	CI10–9	pPKL9, pBeloBAC11(T-6N)	This study
LS68	Δ*fimH*	CI10–9	pPKL9, pBeloBAC11(T-6N/V-10I)	This study
LS154	Δ*fimH*	CI10–9	pPKL9, pBeloBAC11(T-6N/V-10A)	This study
LS141	Δ*fimH*	CI10–9	pPKL9, pBeloBAC11(V-4E)	This study

pJDT3 plasmids contain the parenthetically indicated *fimH* signal sequence allele upstream of the alkaline phosphatase (*phoA*) mature peptide sequence. pBeloBAC11 plasmids contain full-length *fimH* with the parenthetically indicated signal sequence allele and consensus mature peptide sequence.

1. Lee E, Manoil C (1994) Mutations eliminating the protein export function of a membrane-spanning sequence. *J Biol Chem* 269:28822–28828.
2. Sokurenko EV, *et al.* (1998) Pathogenic adaptation of *Escherichia coli* by natural variation of the FimH adhesin. *Proc Natl Acad Sci USA* 95:8922–8926.