Supporting Information

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SI Materials and Methods

Bacterial Strains and Plasmids. To study CsgA mutants for in vivo polymerization, PCR products containing CsgA mutations and NcoI and BamHI restriction endonuclease sites at 5′ and 3′ ends were obtained using standard overlapping PCR extension. PCR products were cloned into NcoI/BamHI sites of vector pLR2 that are downstream of the *csgBA* promoter. To purify CsgA and CsgA* from cell pellets, constructs pNH11 and pNH12 encoding His_6-tagged CsgA and CsgA* without periplasmic Sec signal sequence were generated by standard PCR extension. The PCR product containing NcoI and BamHI sites at 5′ and 3′ ends was cloned into NcoI/BamHI sites downstream to T7 promoter in pET11d. For the test of potential cytoxicity, the empty vector pNH3, pMC3 (pCsgAtrc) containing *C*-terminal His_6-tagged full-length CsgA under *trc* promoter, and $pCsgA^{slowgo}$trc containing $CsgA^{slowgo}$ sequence were generated previously (1–3). pCsgA*trc was constructed by replacing His_6-tagged CsgA with His_6-tagged CsgA* in pMC3.

Purification of CsgA* and CsgA. NEB C2566 cells harboring a pET11d vector encoding His-tagged CsgA (pNH11) or CsgA* (pNH12) were grown to OD_{600} 0.9 in LB broth containing 100 μg/mL ampicillin. The expression of CsgA or CsgA* was induced with 0.5 mM IPTG and induction proceeded at 37°C for 1 h. Cells were collected by centrifugation, and the pellets were stored at −80°C. The cells were resuspended and lysed by extraction solution (8 M GdnHCl, 50 mM K_2HPO_4/KH_2PO_4, pH 7.2). A total of 50 mL of extraction solution was used for cell pellets from a 500-mL culture. The lysate was incubated at 4°C with magnetic stirring for 48 h. The insoluble portion of the lysate was removed by centrifuging at 10,000 × *g*, and the supernatant was incubated with 1.2 mL His-select High Flow nickel-nitrilotriacetic acid resin (NiNTA; Sigma) for 1 h at room temperature. NiNTA beads that bound His-tagged CsgA or CsgA* were loaded on the column, and lysis supernatant passed through the column. GdnHCl in NiNTA was washed away by 12 mL potassium phosphate buffer (50 mM K_2HPO_4/KH_2PO_4, pH 7.2). CsgA or CsgA* was eluted from the column by 0.5 M imidazole and 50 mM potassium phosphate buffer (pH 7.2). Purified proteins were passed through a 30-kDa cutoff filter (Amicon Ultra) to remove possible aggregates and seeds that might alter the polymerization kinetics. His-tagged CsgA and CsgA* showed no difference to CsgA and CsgA* without a His tag in curli assembly in vivo. Therefore, CsgA*-His and CsgA-His are referred to as CsgA* and CsgA for in vitro polymerization study throughout this report.

Preparation of Peptides. All peptides were synthesized by Proteintech Group Inc. Purity was greater than 90% by HPLC, and size was confirmed by mass spectroscopy. Peptides were dissolved to 0.5 mg/mL in trifluoroacetic acid/hexafluoroisopropanol (1:1) and sonicated for 10 min. The suspensions were incubated at room temperature for 1 h. The trifluoroacetic acid/hexafluoroisopropanol was removed by vacuum at room temperature. Peptides were then dissolved in DMSO to a concentration of 10 mg/mL. Freshly prepared peptides were diluted with cold potassium phosphate buffer (50 mM, pH 7.2) for polymerization assay.

ThT Assay. Purified proteins or prepared peptides were loaded on a 96-well opaque plate. ThT was added to a concentration of 20 μM. Fluorescence was measured every 10 min after shaking 5 s by a SpectraMax M2 plate reader (Molecular Devices) set to 438 nm excitation and 495 nm emission with a 475-nm cutoff. ThT fluorescence for the polymerization of CsgA and CsgA mutants was normalized by $(F_i-F_0)/(F_{max}-F_0)$ (4). F_i was the ThT intensity (fluorescence arbitrary unit) of samples, and F_0 was the ThT background intensity. For the measurements of polymerization at different concentrations, F_{max} was the maximum ThT intensity of samples at the highest concentration.

Preparation of Seeds and Seeding Analysis. All peptides (1 mg/mL for R1, R3, and R5; 2 mg/mL for R2 and R4) were incubated for 2 wk at room temperature. R1, R3, and R5 showed strong ThT fluorescence, whereas R2 and R4 did not (5). There were some fibrous structures of R2 and R4 formed as detected by electron microscopy. Solutions containing fibers were sonicated on ice by a Fisher Model 100 sonic dismembrator (Fisher) for three 15-s bursts. Sonications were performed right before the seeding experiment. One to five percent of seeds (by weight) were added to freshly purified or prepared samples immediately before the start of ThT fluorescence assay. The positive seeding result in this manuscript is indicated by the elimination of lag phase of the polymerization process in the presence of less than 5% seeds by weight as measured by ThT fluorescence. R5 peptide polymerization is very fast without significant lag phase, and seeding analysis was performed in the presence 0.5 M or 1.0 M guanidine hydrochloride to slow down its polymerization as previously described (4).

1. Chapman MR, et al. (2002) Role of *Escherichia coli* curli operons in directing amyloid fiber formation. *Science* 295:851–855.
2. Hammer ND, Schmidt JC, Chapman MR (2007) The curli nucleator protein, CsgB, contains an amyloidogenic domain that directs CsgA polymerization. *Proc Natl Acad Sci USA* 104:12494–12499.
3. Wang X, Chapman MR (2008) Sequence determinants of bacterial amyloid formation. *J Mol Biol* 380:570–580.
4. Wang X, Hammer ND, Chapman MR (2008) The molecular basis of functional bacterial amyloid polymerization and nucleation. *J Biol Chem* 283:21530–21539.
5. Wang X, Smith DR, Jones JW, Chapman MR (2007) In vitro polymerization of a functional Escherichia coli amyloid protein. *J Biol Chem* 282:3713–3719.
6. Robinson LS, Ashman EM, Hultgren SJ, Chapman MR (2006) Secretion of curli fibre subunits is mediated by the outer membrane-localized CsgG protein. *Mol Microbiol* 59:870–881.

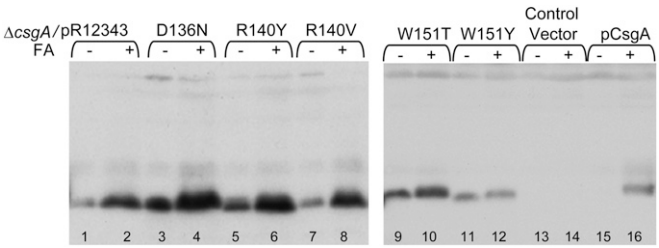

Fig. S1. Western blots of cells and underlying agar from csgA cells containing constructs pR12343 (lanes 1 and 2), pR12343D136N (lanes 3 and 4), pR12343R140Y (lanes 5 and 6), pR12343R140V (lanes 7 and 8), pR12343W151T (lanes 9 and 10), pR12343W151Y (lanes 11 and 12), control vector (lanes 13 and 14), and pCsgA (lane 15 and 16) were grown 48 h at 26°C on YESCA plates. Samples were treated with (+) or without (–) FA as indicated. The blots were probed with anti-CsgA antibody.

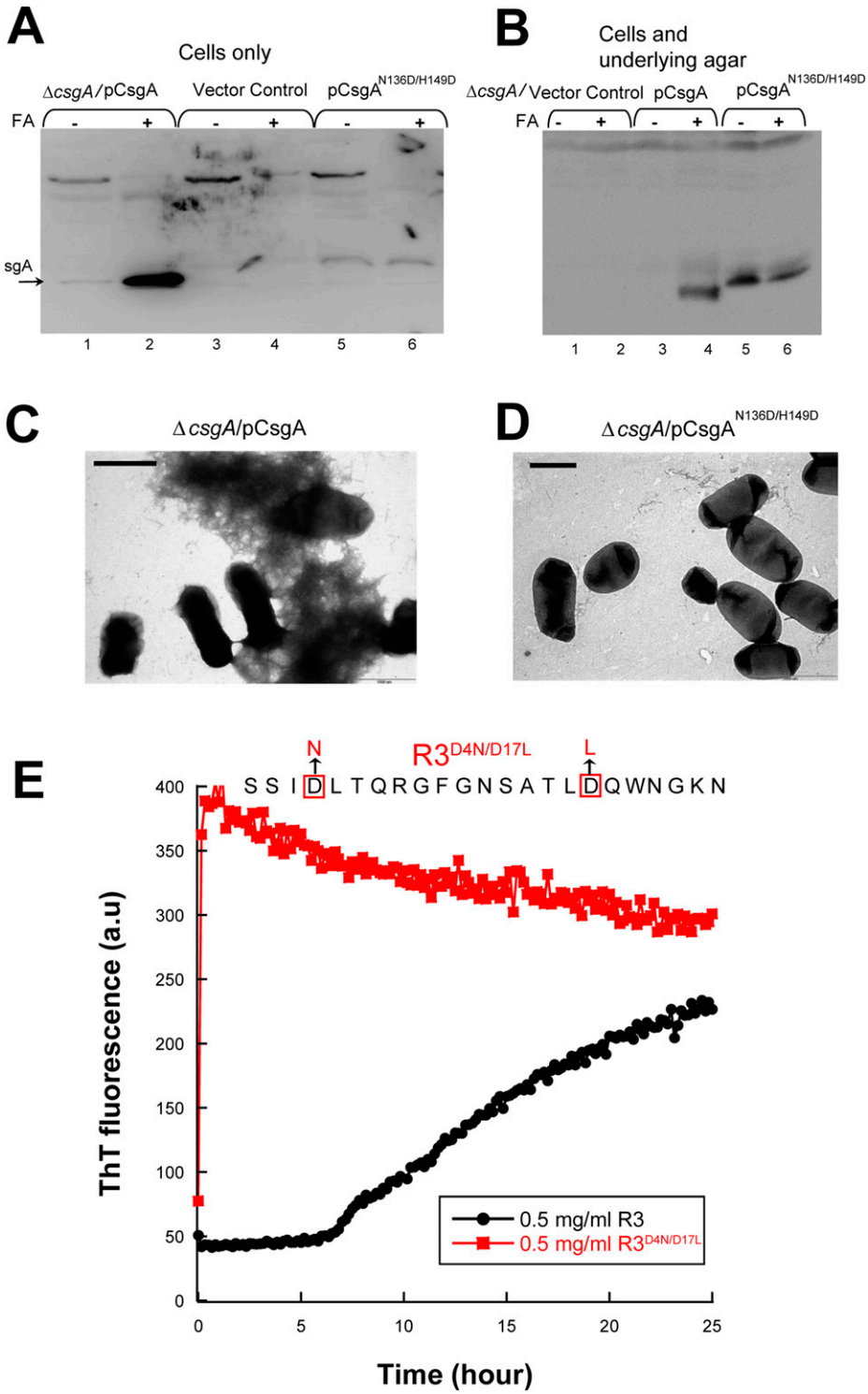

Fig. S2. Gatekeeper residues can disrupt R5 amyloidogenic properties. (*A*) Western blots of whole-cell lysates of *csgA* cells containing constructs pCsgA (lanes 1 and 2), control vector (lanes 3 and 4), and pCsgA$^{N136D/H149D}$ (lanes 5 and 6) were grown 48 h at 26°C on YESCA plates. (*B*) Western blots of cells and underlying agar from *csgA* cells containing constructs control vector (lanes 1 and 2), pCsgA (lanes 3 and 4), and pCsgA$^{N136D/H149D}$ (lanes 5 and 6) were grown 48 h at 26°C on YESCA plates. Samples were treated with (+) or without (–) FA as indicated. The blots were probed with anti-CsgA antibody. Negative-stain EM micrographs of *csgA* cells contain plasmids pCsgA (*C*) or pCsgA$^{N136D/H149D}$ (*D*). Cells were grown 48 h at 26°C on YESCA plates. (Scale bar, 1 μm.) (*E*) The polymerization of 0.5 mg/mL chemically synthesized peptides R3 and R3 $^{D4N/D17L}$ was measured by ThT fluorescence. The changes of amino acid sequence of derivative peptide R3$^{D4N/D17L}$ from R3 are indicated on the top of the graph.

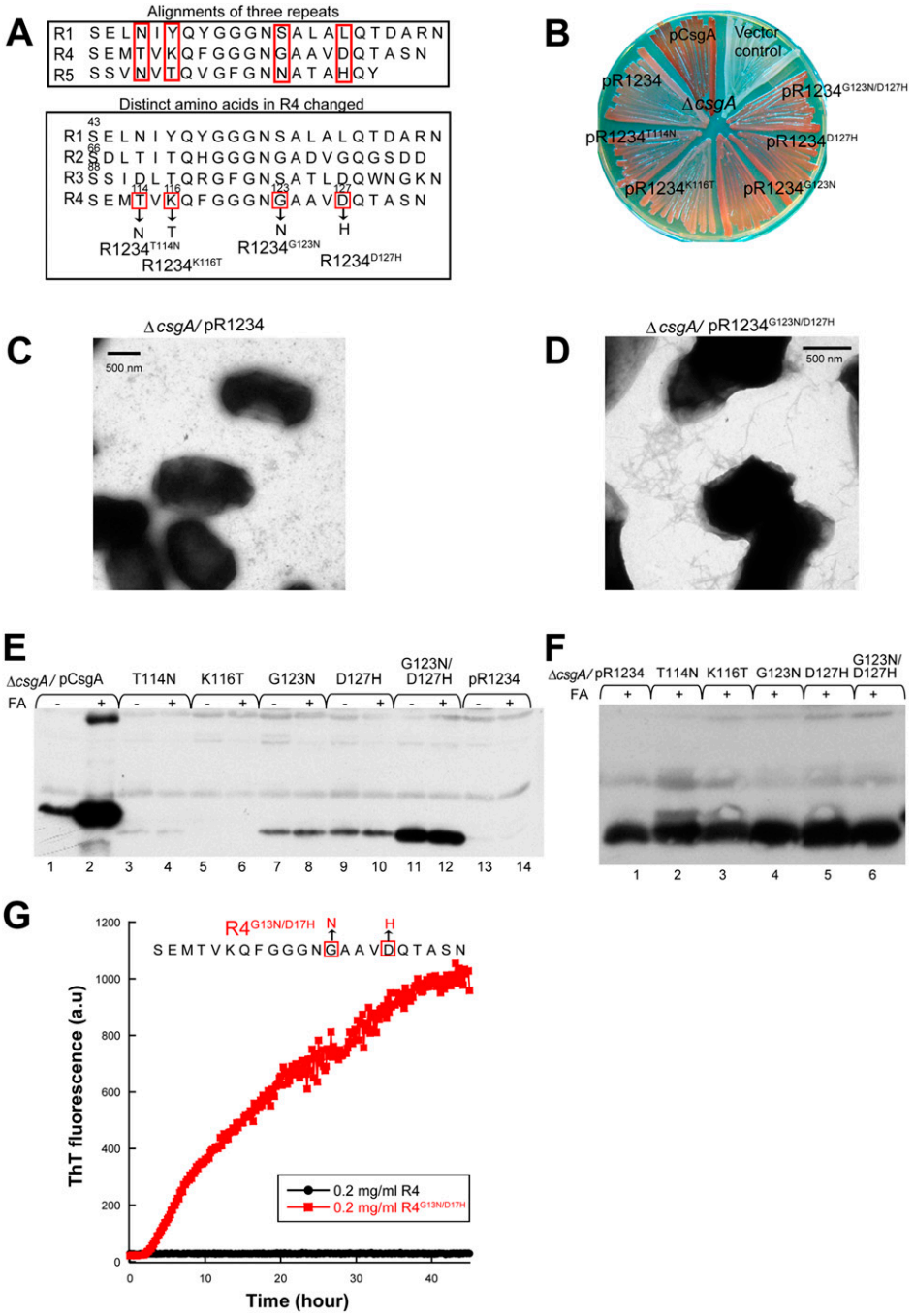

Fig. S3. Identification of gatekeeper residues of R4. (*A*) Differences between R4 and R1/R5 amino acid sequence are indicated with red boxes in *Top*. Constructs with mutations in R4 of R1234 are indicated in *Bottom*. (*B*) Congo red YESCA plate with *csgA* cells transformed with the control vector or plasmids encoding CsgA (pCsgA), R1234, R1234^{T114N}, R1234^{K116T}, R1234^{G123N}, R1234^{D127H}, or R1234$^{G123N/D127H}$. Negative-stain EM micrographs of *csgA* cells containing plasmids pR1234 (*C*) or pR1234$^{G123N/D127H}$ (*D*). Cells were grown 48 h at 26°C on YESCA plates. (Scale bar, 500 nm.) (*E*) Western blots of whole-cell lysates of *csgA* cells containing constructs pCsgA (lanes 1 and 2), pR1234^{T114N} (lanes 3 and 4), pR1234^{K116T} (lanes 5 and 6), pR1234^{G123N} (lanes 7 and 8), pR1234^{D127H} (lanes 9 and 10), pR1234$^{G123N/D127H}$ (lanes 11 and 12), or pR1234 (lanes 13 and 14) grown 48 h at 26°C on YESCA plates. Samples were treated with (+) or without (–) FA as indicated. The blots were probed with anti-CsgA antibody. (*F*) Western blots of cells and underlying agar from *csgA* cells containing constructs pR1234 (lane 1), pR1234^{T114N} (lane 2), pR1234^{K116T} (lane 3), pR1234^{G123N} (lane 4), pR1234^{D127H} (lane 5), or pR1234$^{G123N/D127H}$ (lane 6) grown 48 h at 26°C on YESCA plates. Samples were treated with (+) FA as indicated. The blots were probed with anti-CsgA antibody. (*G*) The polymerization of 0.2-mg/mL chemically synthesized peptides R4 and R4$^{G13N/D17H}$ was measured by ThT fluorescence. The changes of amino acid sequence of derivative peptide R4 $^{G13N/D17H}$ from R4 are indicated on the top of the graph.

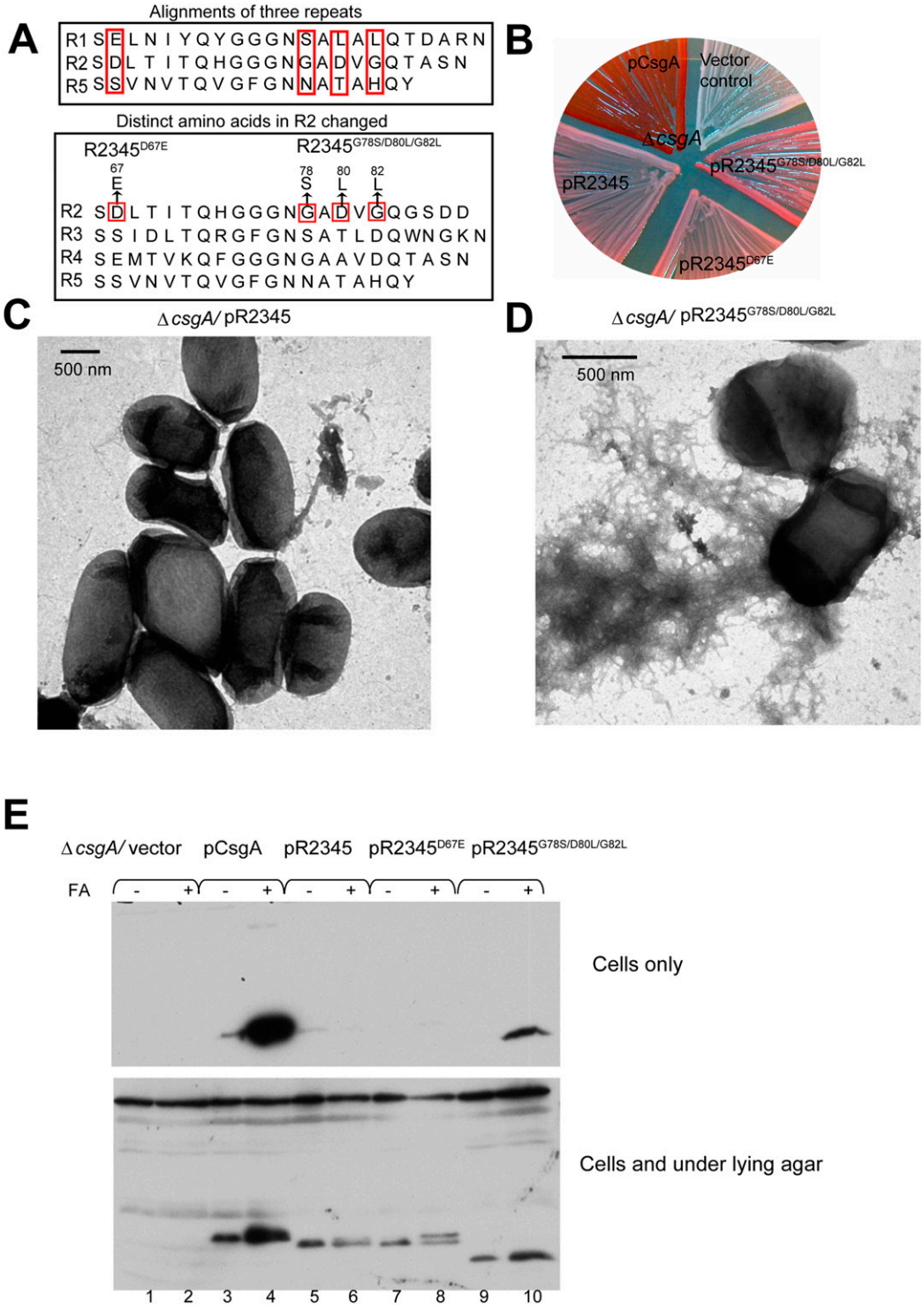

Fig. S4. Identification of gatekeeper residues of R2. (*A*) Differences between R2 and R1/R5 amino acid sequence are indicated with red boxes in *Top*. Constructs with the mutations in R2 of R2345 are indicated in *Bottom*. (*B*) Congo red YESCA plate with *csgA* cells transformed with the control vector or plasmids encoding CsgA, R2345, R2345^{D67E}, or R2345$^{G78S/D80L/G82L}$. The position of amino acids is defined in wild-type CsgA. Negative-stain EM micrographs of *csgA* cells containing plasmids pR2345 (*C*) or pR2345$^{G78S/D80L/G82L}$ (*D*). Cells were grown 48 h at 26°C on YESCA plates. (Scale bar, 500 nm.) (*E*) Western blots of whole-cell lysates (*Top*) or plugs (cells and underlying agar; *Bottom*) from *csgA* mutant cells containing plasmids vector control (lanes 1 and 2), pCsgA (lanes 3 and 4), pR2345 (lanes 5 and 6), pR2345^{D67E} (lanes 7 and 8), or pR2345 $^{G78S/D80L/G82L}$ (lanes 9 and 10) grown 48 h at 26°C on YESCA plates. Cells were treated with (+) or without (–) FA before electrophoresis as indicated. The blots were probed with anti-CsgA antibody.

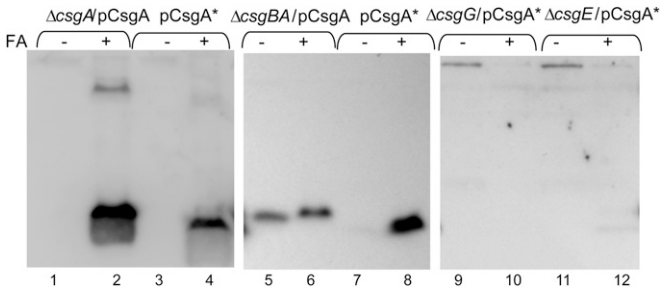

Fig. S5. Western analysis of CsgA and CsgA* in different curli-specific gene deletion backgrounds. Western blot of whole cells and underlying agar (agar plugs) from *csgA*, *csgBA*, *csgG*, and *csgE* cells containing pCsgA or pCsgA*. The blots were probed with anti-CsgA antibody. Lanes 1 and 2 contain *csgA*/pCsgA cells, and lanes 3 and 4 contain *csgA*/pCsgA*. Lanes 5 and 6 contain *csgBA*/pCsgA cells, and lanes 7 and 8 contain *csgBA*/pCsgA* cells. Lanes 9 and 10 contain *csgG*/pCsgA*, and lanes 11 and 12 contain *csgE*/pCsgA*. Samples were treated with (+) or without (–) FA as indicated. The blots were probed with anti-CsgA antibody.

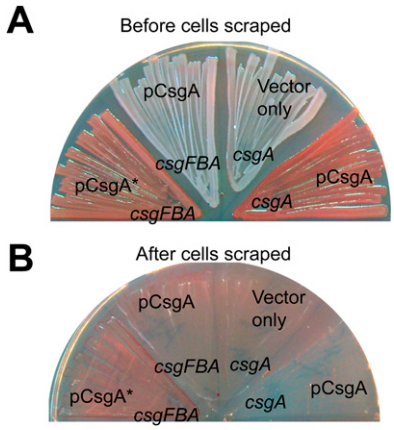

Fig. S6. Mislocalized CsgA* fibers were formed as measured on Congo red plates after 48 h of growth. (*A*) A Congo red-containing YESCA plate with *csgA* transformed with control vector or pCsgA and *csgFBA* transformed with pCsgA or pCsgA*. Cells were grown 48 h at 26°C. (*B*) Cells were mechanically scraped off of a Congo red-containing YESCA plate using disposable inoculating loops to compare the Congo red binding phenotypes of *csgA*/pLR2, *csgA*/pCsgA, *csgFBA*/pCsgA, and *csgFBA*/pCsgA*.

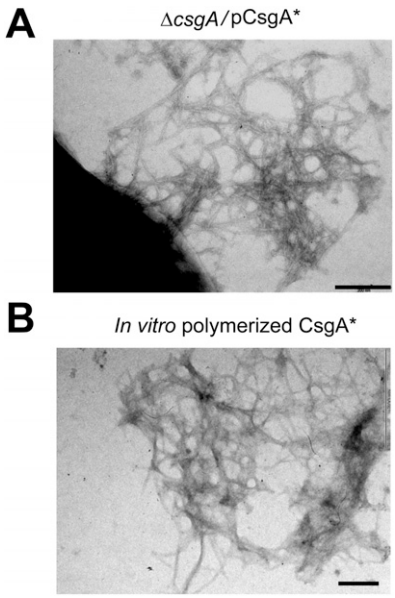

Fig. S7. Negative-stain EM micrographs of polymerized CsgA*. (*A*) Negative-stain EM micrographs of *csgA* cells containing plasmids pCsgA*. Cells were grown 48 h at 26°C on YESCA plates. (*B*) In vitro polymerized fibers of CsgA*. (Scale bars, 200 nm.)

Table S1. Summary of the curli-assembly phenotype of CsgA derivatives

Strains	Congo red staining[†]	Fibers by TEM[‡]	Cell association[§]	Fiber SDS solubility[¶]
MC4100 (wild-type)	++++	++++	+++	Insoluble
csgA/Empty vector	–	–	–	—
csgA/pCsgA	++++	++++	+++	Insoluble
csgA/pR12341	++++	++++	+++	Insoluble
csgA/pR52345	+++	++++	+++	Insoluble
csgA/pR12343	++	–	–	—
csgA/pR32345	++	+	++	Soluble
csgBA/pR12341	–	–	–	—
csgBA/pR52345	–	–	–	—
csgA/pR12343^{D136N}	+++	++	+	Partially insoluble
csgA/pR12343^{R140Y}	++	–	–	—
csgA/pR12343^{R140V}	++	–	–	—
csgA/pR12343^{D149L}	++++	+++	++	Partially insoluble
csgA/pR12343^{D149H}	++++	+++	++	Partially insoluble
csgA/pR12343^{W151T}	++	–	–	—
csgA/pR12343^{W151Y}	++	–	–	—
csgA/pR12343^{D149R}	++++	+++	++	Partially insoluble
csgA/pR12343^{D149K}	++++	+++	++	Partially insoluble
csgA/pCsgA$^{N136D/H149D}$	++	–	–	—
csgA/pR1234	++	–	–	—
csgA/pR1234^{T114N}	++	–	–	—
csgA/pR1234^{K116T}	++	–	–	—
csgA/pR1234^{G123N}	+++	++	+	Soluble
csgA/pR1234^{D127H}	+++	++	+	Soluble
csgA/pR1234G$^{123N/D127H}$	+++	+++	++	Soluble
csgA/pR2345	+	–	–	—
csgA/pR2345^{D67E}	+	–	–	—
csgA/pR2345$^{G78S/D80L/G82L}$	+++	++++	++	Insoluble
csgA/pCsgA*	++++	++++	+++	Insoluble
csgBA/pCsgA	–	–	–	–
csgBA/pCsgA*	++++	++++	++	Insoluble
csgFBA/pCsgA	–	–	–	—
csgFBA/pCsgA*	+++	++++	++	Insoluble
csgFB/pCsgA	–	–	–	—
csgFB/pCsgA*	++++	++++	++	Insoluble
csgG/pCsgA	–	–	–	—
csgG/pCsgA*	–	–	–	—
csgE/pCsgA	–	–	–	—
csgE/pCsgA*	–	–	–	—

[†]Congo red staining was scored by the color of cells grown on Congo red YESCA plates for 48 h at 26°C. The color was scored as follows: ++++, red as wild type; +++, dark pink; ++, pink; +, slight pink; –, white.

[‡]TEM analysis of fiber formation was scored as follows: ++++, wild type; +++, slightly reduced levels; ++, significantly reduced level; +, few fibers; –, none detected.

[§]Cell association was measured by comparing the protein level of cells and comparing cells with agars. It scored as follows: +++, wild type; ++, slightly reduced levels; +, significantly reduced level by whole-cell Western analysis; –, no significant band detected by whole-cells Western analysis.

[¶]SDS solubility was measured by the protein level of samples without FA treatment by Western analysis of whole-cell lysates. The — indicates that there is no significant band detected by whole-cell Western analysis.

Table S2. Strains and plasmids used in this study

Strains and plasmids	Relevant characteristics	References
Strains		
csgA (LSR10)	MC4100 Δ*csgA*	1
csg*BA* (LSR13)	MC4100 Δ*csgBA*	2
csgG (LSR1)	MC4100 *csgG*::Tn105	6
csgE (MHR480)	MC4100 Δ*csgE*	1
csgFB (MHR442)	MC4100 Δ*csgFB*	2
csgFBA (NDH108)	*csgF* Δ*csgBA*	2
NEB #C2566	T7 express competent *E. coli* (high efficiency)	NEB Inc.
LSR12	C600 *csgBA and csgDEFG*	1
Plasmids		
pCsgA (pLR5)	*csgA* sequence in pLR2	4
pLR2	control vector containing *csgBA* promoter	6
pR12341	CsgA repeat 5 replaced by 1 in pLR2	In the text
pR52345	CsgA repeat 1 (residues 43–61) replaced by repeat 5 in pLR2	In the text
pR32345	CsgA repeat 1 replaced by 3 in pLR2	In the text
pR12343	CsgA repeat 5 replaced by 3 in pLR2	In the text
pR12343^{D136N} (pXW56)	D136N in R12343 in pLR2	In the text
pR12343^{R140Y} (pXW57)	R140Y in R12343 in pLR2	In the text
pR12343^{R140V} (pXW58)	R140V in R12343 in pLR2	In the text
pR12343^{D149L} (pXW59)	D149L in R12343 in pLR2	In the text
pR12343^{D149H} (pXW60)	D149H in R12343 in pLR2	In the text
pR12343^{W151T} (pXW61)	W151T in R12343 in pLR2	In the text
pR12343^{W151Y} (pXW62)	W151Y in R12343 in pLR2	In the text
pCsgA*	G78S/D80L/G82L/D91N/D104L	In the text
(pXW86)	/G123N/D127H in CsgA in pLR2	—
pR1234 (pΔR5)	*csgA* without R5 (S^{133} to Y^{151}) in pLR2	4
pR1234^{T114N} (pYZ1)	T114N in R1234 in pLR2	In the text
pR1234^{K116T} (pYZ2)	K116T in R1234 in pLR2	In the text
pR1234^{G123N} (pYZ3)	G123N in R1234 in pLR2	In the text
pR1234^{D127H} (pYZ4)	D127H in R1234 in pLR2	In the text
pR1234$^{G123N/D127H}$	G123N and D127H in R1234 in pLR2	In the text
(pYZ12)		—
pR2345 (pΔR1)	*csgA* without R1 (S^{43} to S^{65}) in pLR2	4
pR2345^{D67E} (pYZ9)	D67E in R2345 in pLR2	In the text
pR2345$^{G78S/D80L/G82L}$	G78S/D80L/G82L in R2345 in pLR2	In the text
(pYZ10)		—
pCsgA$^{N136D/H149D}$	N136D/H149D in CsgA in pLR2	In the text
(pXW81)		—
pMC3 (pCsgAtrc)	Sequence encoding His-tagged *csgA* cloned into the IPTG-inducible plasmid pHL3	1
pCsgA^{slowgo}trc	Sequence encoding His-tagged CsgA$^{Q49A/N54A/Q139A/N144A}$ cloned into pHL3	3
pNH3	Empty expression vector for pCsgAtrc	2
pCsgA*trc	Sequence encoding His-tagged CsgA $^{G78S/D80L/G82L/D91N/D104L/G123N/D127H}$ cloned into pHL3	In the text
pNH11	Sequence encoding CsgA without Sec-signal peptide (residues 1–20) cloned into pET11d under T7 promoter	In the text
pNH12	Sequence encoding CsgA* without Sec-signal peptide (residues 1–20) cloned into pET 11d under T7 promoter	In the text

Table S3. Sequence of primers used in this study

Primer Name	Sequence
FpLR5[a]	5′ CATGCCATGGCGAAACTTTTAAAAGTAGC 3′
RpLR5[b]	5′ CGGGATCCTGTATTAGTACTGAT 3′
pR12341P1	5′ GTTCAGCTCAGAGTTAGATGCAGTCTG 3′
pR12341P2	5′ CAGACTGCATCTAACTCTGAGCTGAAC 3′
pR12341P3	5′ CGGGATCCTTAGTTACGGGCATCAG 3′
pR52345 P1	5′ CGTTGACGGAGGAATTTGGGCCGCTATT 3′
pR52345 P2	5′ AATAGCGGCCCAAATTCCTCCGTCAACG 3′
pR52345 P3	5′ AGTCAAGTCAGAGTTACGGGCATCGTACTGATGAGCG 3′
pR52345 P4	5′ CGCTCATCAGTACGATGCCCGTAACTCTGACTTGACT 3′
pR32345 P1	5′ GGGTAATAGTCAAGTCAGAATTTTTGCCGTTCCACTGATC 3′
pR32345 P2	5′ GATCAGTGGAACGGCAAAAATTCTGACTTGACTATTACCC 3′
pR12343 P1	5′ CAGATCGATTGAGCTGTTAGATGCAGTCTG 3′
pR12343 P2	5′ CAGACTGCATCTAACAGCTCAATCGATCTG 3′
pR12343 P3	5′ CGGGATCCTTAATTTTTGCCGTTCCA 3′
pXW56 P2	5′ CGGGATCCTTAATTTTTGCCGTTCCACTGATCAAGAGTAGCGCTGTTACCGAAGCCACGTTGGGTCAGATTGATTGAGCTGTT AGA 3′
pXW57 P2	5′ CGGGATCCTTAATTTTTGCCGTTCCACTGATCAAGA GTAGCGCTGTTACCGAAGCCGTATTGGGTCAGATCG 3′
pXW58 P2	5′ CGGGATCCTTAATTTTTGCCGTTCCACTGATCAAGAGTAGCGCTGTTACCGAAGCCAACTTGGGTCAGATCG3′
pXW59 P2	5′ CGGGATCCTTAATTTTTGCCGTTCCACTGATGAAGAGTAGCGCTG 3′
pXW60 P2	5′ CGGGATCCTTAATTTTTGCCGTTCCACTGAAGAAGAGTAGCGCTG 3′
pXW61 P2	5′ CGGGATCCTTAATTTTTGCCGTTGGTCTGATCAAGAGTAG 3′
pXW62 P2	5′ CGGGATCCTTAATTTTTGCCGTTGTACTGATCAAGAGTAG 3′
pCsgA* P1	5′ GCCACGTTGGGTCAGGTTGATTGAGCTGTCATCTGAGCCCTGCAGAACCAGTGCAGAATTACCGCCGCCATG 3′
pCsgA* P2	5′ CATGGCGGCGGTAATTCTGCACTGGTTCTGCAGGGCTCAGATGACAGCTCAATCAACCTGACCCAACGTGGC 3′
pCsgA* P3	5′ GTTAGATGCAGTCTGATGAACTGCAGCGTTGTTGCCACCACCGAAC 3′
pCsgA* P4	5′ GTTCGGTGGTGGCAACAACGCTGCAGTTCATCAGACTGCATCTAAC 3′
pXW81 P1	5′ CTGAGTCACGTCGACGGAGGAG 3′
pXW81 P2	5′ CTCCTCCGTCGACGTGACTCAG 3′
pYZ1 P1	5′ CGGGATCCTTAGTTAGATGCAGTCTGGTCAACTGCAGCACCGTTGCCACCACCGAACTGTTTAACGTTCATTTCAGA 3′
pYZ2 P1	5′CGGGATCCTTAGTTAGATGCAGTCTGGTCAACTGCAGCACCGTTGCCACCACCGAACTGAGTAACCGTCATTTCAG3′
pYZ3 P1	5′ CGGGATCCTTAGTTAGATGCAGTCTGGTCAACTGCAGCGTTGTTGCCACCACCG 3′
pYZ4 P1	5′ CGGGATCCTTAGTTAGATGCAGTCTGATGAACTGCAGCACCG 3′
pYZ12 P1	5′ CGGGATCCTTAGTTAGATGCAGTCTGATGAACTGCAGCG TTGTTGCCACCACCG 3′
pYZ9 P1	5′ GGTAATAGTCAACTCAGAATTTGGGCCG 3′
pYZ9 P2	5′ CGGCCCAAATTCTGAGTTGACTATTACC 3′
pYZ10 P1	5′ GTCATCTGAGCCCTGCAGAACAAGTGCAGAATTACCGCCG 3′
pYZ10P2	5′ CGGCGGTAATTCTGCACTTGTTCTGCAGGGCTCAGATGAC 3′
NDH55 [c]	5′ GCGTTTCCATGGGTGTTGTTCCTCAGTACGGCGGC 3′
NDH56 [c]	5′ GTTTAAAGCTTGGATCCTTAGTGATGGTGATGGTGATGGTACTGATGAGCGGTCGCGTTGTT 3′

[a]FpLR5 is paired to noncoding strand immediately upstream of the start codon of *csgA* in pLR5.
[b]RpLR5 is paired to coding strand immediately downstream of the stop codon of *csgA* in pLR5.
[c]NDH55 and NDH56 were used to make constructs pNH11 and pNH12.