Supplementary Information

Cysteine dependent conformation heterogeneity of *Shigella flexneri* autotransporter IcsA and implications in its function

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Table S1. Strains, plasmids, and oligonucleotides

| Bacterial strains | Description | Source |
|-------------------|-------------|--------|
| **S. flexneri strains** | | |
| 2457T | Wild-type Shigella flexneri 2a | 1 |
| ΔicsA | Shigella flexneri 2a icsA::tet | 2 |
| ΔipaD | Shigella flexneri 2a ipaD::frt | 3 |
| ΔicsA ΔipaD | Shigella flexneri 2a icsA::tet ipaD::frt | 3 |
| ΔicsB | Shigella flexneri 2a icsB::kan | This work |
| ΔicsA ΔicsB | Shigella flexneri 2a icsA::tet icsB::kan | This work |
| **E. coli strains** | | |
| DH5a | F– φ80lacZΔM15 Δ(lacZYA-argF)U169 recA1 endA1 hsdR17(rK–, mK+) phoA supE44 λ– thi-1 gyrA96 relA1 | Lab stock |
| TOP10 | F– mcrA Δ(mrr−hsdRMS-mcrBC) φ80lacZΔM15 ΔlacX74 recA1 araD139 Δ(ara-leu)7697 galU galK λ– rpsL(StrR) endA1 nupG | Invitrogen |
| BL21C43(DE3) | F– ompT gal dcm lon hsdSB(rB–mB–) λ(DE3 [lacI lacUV5-T7 gene 1 ind1sam7 nin5]) and two uncharacterized mutations | Lucigen |

| Plasmids | | |
|----------|-------------|--------|
| plcsA | icsA with its native promoter cloned into pBR322 | 4 |
| pBR322 | Cloning vector, AmpR, TcR | 5 |
| pKD46 | Temperature sensitive plasmid expressing Red proteins, AmpR | 6 |
| pKD4 | Plasmid carrying FRT flanked kanamycin resistant cassette, AmpR, KanR | 6 |
| plcsB-lpgA | icsB and lpgA with its native promoter cloned into pSU2718 | This work |
| pSU2718 | Cloning vector, ChlR | 7 |
| plcsA737-FLAG | FLAG×3 affinity tag inserted at i737 in plcsA | This work |
| plcsA-lcsP | icsA and lcsP cloned into pCDFDuet-1 with FLAG×3 affinity tag inserted at i54 of icsA | This work |
| plcsA130S | Codon substitution of cysteine to serine at 130 | This work |
| plcsA375S | Codon substitution of cysteine to serine at 375 | This work |
| plcsA379S | Codon substitution of cysteine to serine at 379 | This work |
| plcsA375S/C379S | Codon substitution of cysteine to serine at 375 and 379 | This work |
| plcsAFLAG-C130S | FLAG×3 in-frame addition at i737 in plcsA130S | This work |
| plcsAFLAG-C375S | FLAG×3 in-frame addition at i737 in plcsA375S | This work |
| plcsAFLAG-C379S | FLAG×3 in-frame addition at i737 in plcsA379S | This work |
| plcsAFLAG-C375S/C379S | FLAG×3 in-frame addition at i737 in plcsA375S/C379S | This work |

| Oligos | | |
|--------|-------------|--------|
| Description | Sequence |
| NcoI-icsA Fwd | CTACGACCATGGCTATGAATCAAATTCACAAATTTTTTGTAATATGACCC |
| icsA-Sall Rev | CTACGAGTCGACTCAGGGGTATATTTTCACACCCAAAATAC |
NdeI-IcsP Fwd
icsP-KpnI Rev
FLAG i54 addition Fwd
FLAG i54 addition Rev
FLAG i737 addition Fwd
FLAG i737 addition Rev
icsB KO Fwd
icsB KO Rev
icsB-ipgA Fwd
icsB-ipgA Rev
IcsA C130S Fwd
IcsA C130S Rev
IcsA C375S/C379S Fwd
IcsA C375S/C379S Rev
IcsA C375S Rev
IcsA C379S Fwd
IcsA C379S Rev
Figure S1. Plaque formation by *S. flexneri* 2457T and its derivatives producing IcsA \(^{WT}\) and mutant IcsA proteins with MDCK-2 cells. Representative images were shown, and data was used to generate Figure 5 d&e respectively.
Figure S2. Western immunoblotting with bacterial cells expressing IcsA and its cysteine substitution mutants from 2457TΔipaD treated with hNE. Samples were taken at different time points as indicated above the blot. Samples from bacteria expressing IcsAWT were electrophoresed in parallel (left) with mutants (right) and blotted together to ensure equal exposure for direct comparison. Molecular markers (Precision Plus protein Dual color standards #1610374 BioRad) shown left as 250 kDa, 150 kDa, 100 kDa, 75 kDa, 50 kDa, 37 kDa, 25 kDa, 20 kDa, 15 kDa, and 10 kDa.

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