

1 **The autotransporter IcsA promotes *Shigella flexneri* biofilm formation in presence of bile**
2 **salts**

3 Volkan K. Köseoğlu¹, Chelsea P. Hall¹, Eric M. Rodríguez-López², Hervé Agaisse^{1,*}

4
5 **Content:**

6 **Figure S1: Bacterial cell-cell interaction observed in TSB-BS.**

7 **Figure S2: TSB-CA biofilms of *S. flexneri* exhibit dramatic variations in thickness.**

8 **Figure S3: Quantification of volume of the biofilms formed by the *icsA* pIcsA-Myc strain.**

9 **Figure S4: IcsA-Myc species in the pellet of biofilm spent media.**

10

11

12

13

14

15

16

17

18

19

20

21

22

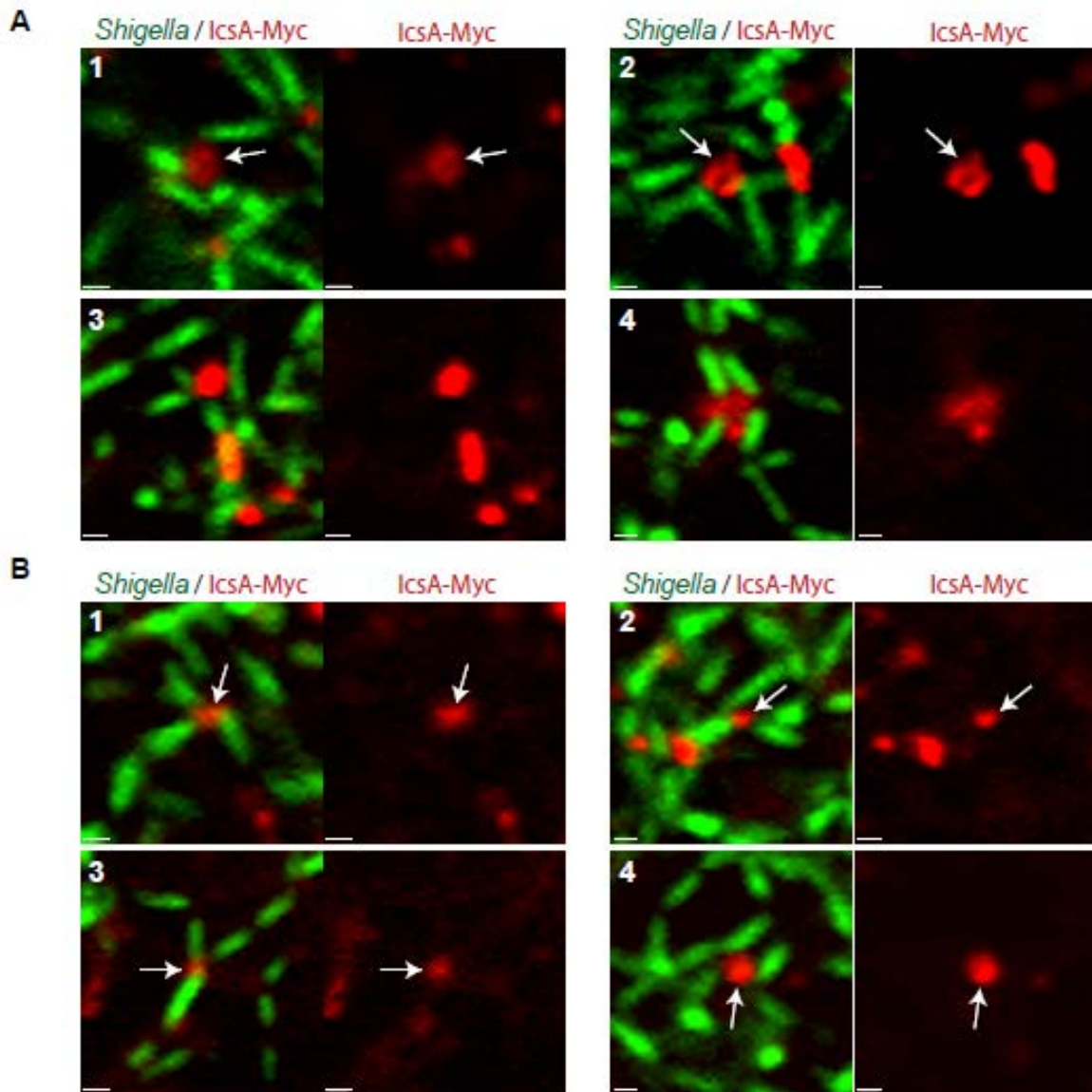
23

24 **Figure S1: Extensive bacterial interaction observed in the biofilm formed in TSB-BS.**

25 **(A)** Representative images (1-4) depicting interactions of 4 bacterial cells around a single IcsA-

26 Myc punctae. **(B)** Representative images (1-4) depicting interactions of 3 bacterial cells around a

27 single IcsA-Myc punctae. Scale bar is 1 μ m. Green, *Shigella*; Red, IcsA-Myc.

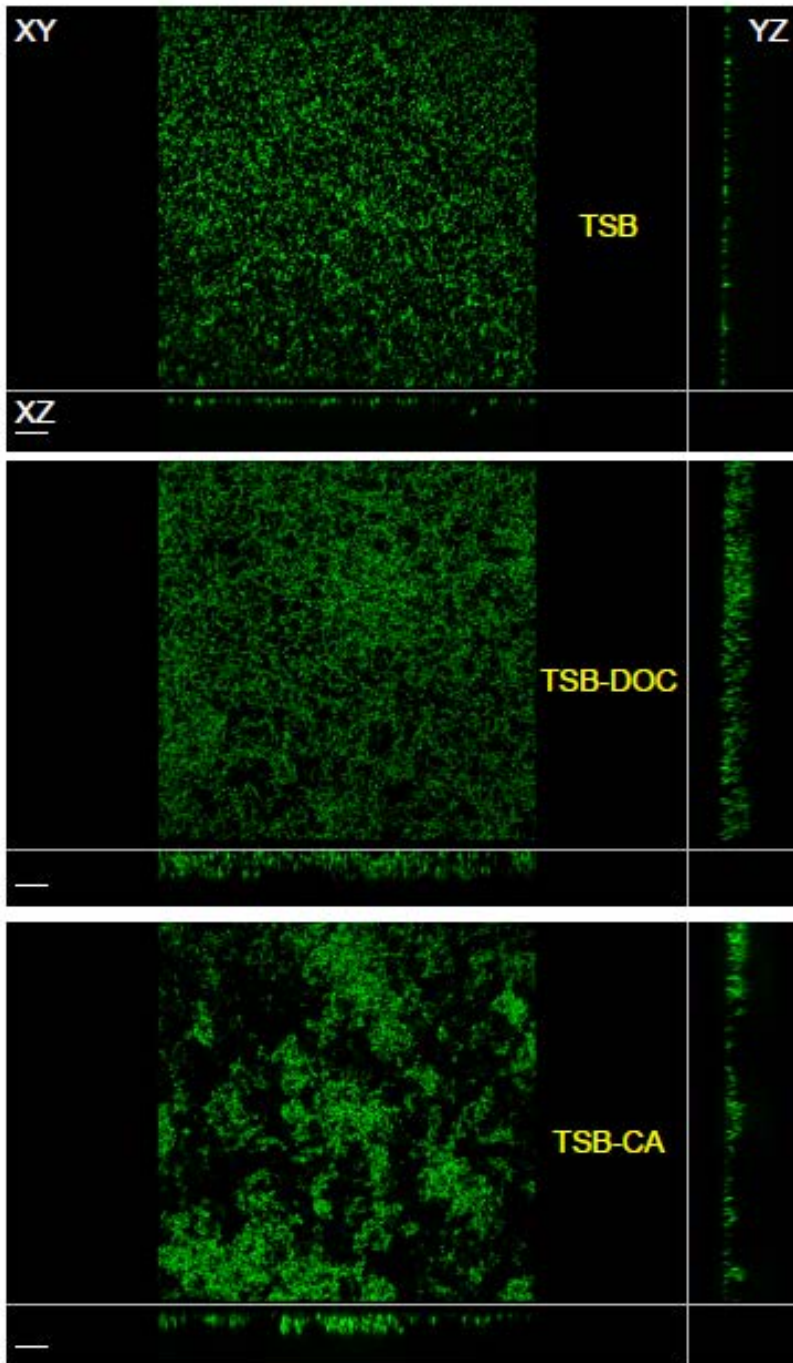


28

29

30

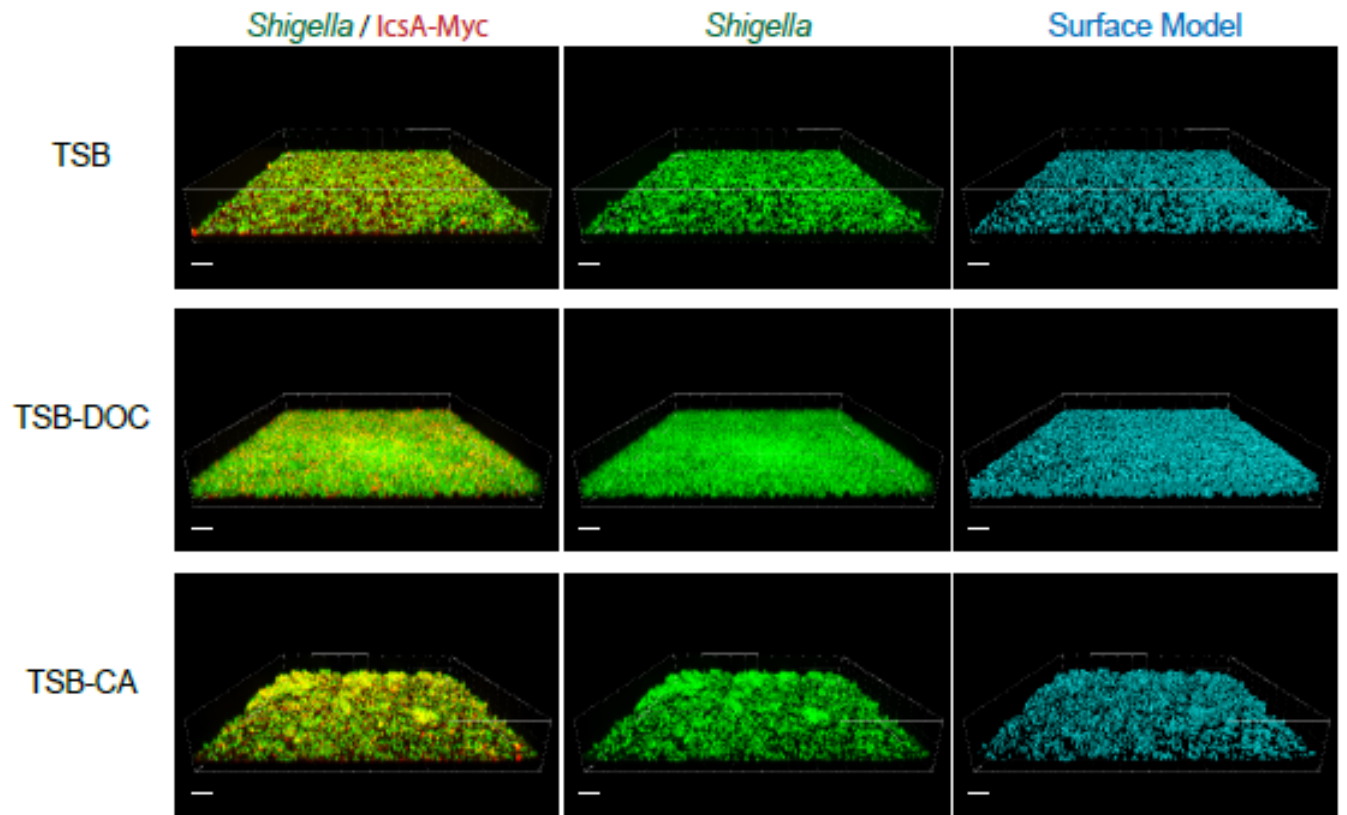
31 **Figure S2: TSB-CA biofilms of *S. flexneri* exhibit dramatic variations in thickness.**
32 Representative XY, YZ, and XZ views from a representative biofilm formed in each medium
33 (TSB, TSB-DOC, and TSB-CA).



34

35

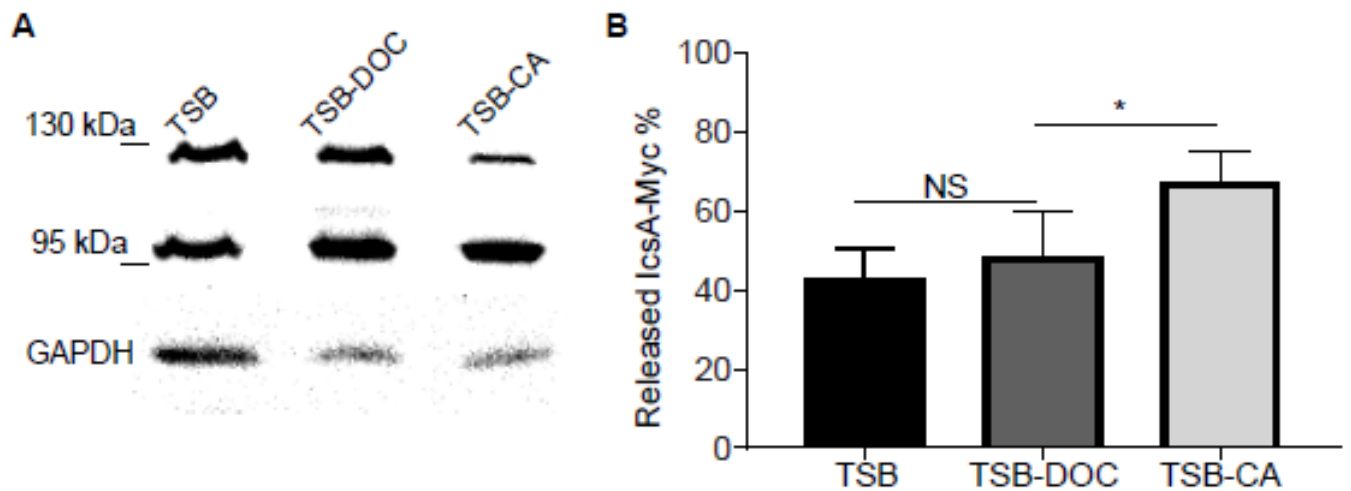
36 **Figure S3: Quantification of the volume of the biofilms formed by the *icsA* pIcsA-Myc**
37 **strain.** Representative 3D images. Green, *S. flexneri*; Red, IcsA-Myc; Blue, Surface model of
38 biofilm based on Green (*S. flexneri*) signal. Scale bar is 20 μm .



39
40
41
42
43
44
45
46
47
48

49 **Figure S4: IcsA-Myc species in the pellet of biofilm spent media.** (A) Western-blot analysis
50 of IcsA-Myc in floating aggregates after biofilm formation in TSB, TSB-DOC and TSB-CA
51 media. Biofilm spent media were collected and centrifuged to separate supernatant and
52 aggregates. Floating aggregates were collected at the bottom of Eppendorf tubes and
53 immediately treated with SDS sample buffer containing DTT. Cleared supernatant was filter-
54 sterilized and TCA precipitated to isolate soluble proteins. No IcsA-Myc species were detected
55 in filtered supernatants (data not shown). (B) Quantification of cleaved IcsA-Myc as a
56 percentage of total IcsA-Myc (intact IcsA + cleaved IcsA). One-way ANOVA with Tukey's
57 multiple comparisons test was applied for statistical analysis using GraphPad Prism 7.02. NS:
58 not significant, *: p -value < 0.05. Error bars indicate standard error of the mean for three
59 biological replicates.

60



61