

1 **Confinement Discerns Swarms from Planktonic Bacteria**

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13 **Author Contribution**

14 W.C., N.M., J.X.T., and S.M. conceived and designed the work. W.C. and N.M. performed the
15 experiments and analyzed the data. H.K. performed the computational simulations. H.L. isolated
16 the bacteria strains and prepared the mouse tissues. W.C., H.K., S.M., and J.X.T. wrote the paper.

17 **Competing interests**

18 Weijie Chen, Neha Mani, Jay X. Tang, and Sridhar Mani filed a U.S. patent application
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20 **Abstract**

21 Powered by flagella, many bacterial species exhibit collective motion on a solid surface commonly
22 known as swarming. As a natural example of active matter, swarming is also an essential biological
23 phenotype associated with virulence, chemotaxis, and host pathogenesis. Physical changes like
24 cell elongation and hyper flagellation have been shown to accompany the swarming phenotype.
25 However, less noticeable, are the contrasts of collective motion between the swarming cells and
26 the planktonic cells of comparable cell density. Here, we show that confining bacterial movement
27 in designed dimensions allows distinguishing bacterial swarming from collective swimming. We
28 found that on a soft agar plate, a novel bacterial strain *Enterobacter* sp. SM3 exhibited different
29 motion patterns in swarming and planktonic states when confined to circular microwells of a
30 specific range of sizes. When the confinement diameter was between 40 μm and 90 μm , swarming
31 SM3 formed a single swirl motion pattern in the microwells whereas planktonic SM3 showed
32 multiple swirls. Similar differential behavior is observed across a range of randomly selected
33 gram-negative bacteria. We hypothesize that the “rafting behavior” of the swarming bacteria upon
34 dilution might account for the motion pattern difference. We verified our conjectures via numerical
35 simulations where swarming cells are modeled with lower repulsion and more substantial
36 alignment force. The novel technical approach enabled us to observe swarming on a non-agar
37 tissue surface for the first time. Our work provides the basis for characterizing bacterial swarming
38 under more sophisticated environments, such as polymicrobial swarmer detection, and *in vivo*
39 swarming exploration.

40

41 Main Text

42 Introduction

43 Motility is an essential characteristic of bacteria. Although energy-consuming, it provides high
44 returns, enabling cells to uptake nutrients efficiently and escape from noxious
45 environments(Webre, Wolanin, & Stock, 2003). In a host environment, bacterial motility is an
46 essential phenotype that intimately relates to virulence through complex regulatory
47 networks(Josenhans & Suerbaum, 2002). Swimming and swarming are two common motility
48 phenotypes mediated by flagella. Whereas the planktonic phenotype defines individual bacteria's
49 motility, a collective movement powered by rotating flagella(Kearns, 2010a) on a partially
50 solidified surface defines swarming(Partridge & Harshey, 2013). In swarming, bacteria utilize
51 their flagella to navigate, two-dimensionally, through a medium and acquire necessary molecules
52 to maintain homeostasis and overall survival(N. C. Darnton, Turner, Rojevsky, & Berg, 2010).
53 Morphological changes like cell elongation may or may not occur in all swarming
54 bacteria(Michaels & Tisa, 2011). Thus, concentrated swimming bacteria are often called "a swarm
55 of bacteria" without requiring precise identification of swarming motility, per se. Nevertheless,
56 microbiologists believe that swarming and swimming are fundamentally different motility types.
57 For instance, studies found that compared with swimming cells, the requirement for flagella torque
58 is higher for swarming *B. subtilis*(Hall, Subramanian, Oshiro, Canzoneri, & Kearns, 2018);
59 swarming *E. coli* remodel their chemotaxis pathway(Partridge, Nhu, Dufour, & Harshey, 2019);
60 and in swarming *P. aeruginosa*, both the production of virulence factors and antibiotic resistance
61 increase(Overhage, Bains, Brazas, & Hancock, 2008). A recent study has demonstrated a
62 medically relevant distinction between swarming and swimming: a particular strain of swarming
63 *Enterobacter* protect against mice intestinal inflammation while their swimming counterparts
64 could not(Chen et al., 2020). The evidence to date that shows swarming is different from
65 swimming comes mostly from biological data(Kearns, 2010b). However, precise biophysical
66 visualization and quantitation of these differences are lacking. In this report, using *Enterobacter*
67 sp. SM3, which is a novel strain that possesses both swimming and swarming motilities, we show
68 distinct biophysical characteristics between these two types of motility under confined, circular
69 geometry of a particular confinement size range.

70 Studies have shown that geometric constraints have a profound influence on patterns of
71 microswimmers' collective motion. For example, these constraints may create mesoscopically or
72 macroscopically coherent structures such as swirls and jets(Theillard, Alonso-Matilla, & Saintillan,
73 2017; Wioland, Lushi, & Goldstein, 2016; Wioland, Woodhouse, Dunkel, & Goldstein, 2016).
74 Circular confinement, in particular, could stabilize a suspension of motile bacteria into a spiral
75 vortex(Beppu et al., 2017; Lushi, Wioland, & Goldstein, 2014; Nishiguchi, Aranson, Snezhko, &
76 Sokolov, 2018; Wioland, Woodhouse, Dunkel, Kessler, & Goldstein, 2013). Here, we compare
77 the behaviors of bacteria in swarming and planktonic states under quasi-two dimension (quasi-2D)
78 circular confinement. Many species of bacteria show distinctive motion patterns while confined.
79 This characteristic may lead to future diagnostic applications since there are established
80 associations between bacterial swarming and virulence pathology(Lane, Alteri, Smith, & Mobley,
81 2007; Overhage et al., 2008).

82 **Results**

83 **Swarming *Enterobacter* sp. SM3 forms large single swirls**

84 A novel bacterial strain *Enterobacter* sp. SM3 (NCBI BioProject PRJNA558971), isolated in 2014
85 from mice with colitis induced with dextran sulfate sodium (DSS), has been previously studied for
86 motility(Araujo, Chen, Mani, & Tang, 2019) and host phenotype(Chen et al., 2020). SM3 expands
87 rapidly on 0.5% agar with the collective motion of multilayers of cells at the edge. We mounted a
88 PDMS chip containing circular microwells on the agar so that bacteria in confinement could rotate
89 for more than 3 hours (details with illustration in Methods). Under confinement in circular wells
90 in the diameter range of 31-90 μm , swarming SM3 shows single swirls. In contrast, SM3
91 planktonic cells concentrated from the liquid medium form mesoscale vortices (multiple swirls) in
92 the same size range, except for the smallest well diameter of 31 μm . A clear difference is shown
93 at the well diameter of 74 μm (Fig. 1A-D, Movie S1 & S2). This striking difference persists in
94 several well depths, except that the concentrated cells yield small but non-zero vortex order
95 parameters (VOPs, defined as illustrated in Fig. 1E) in deeper wells, instead of nearly zero VOPs
96 in shallow wells (Fig. 1F).

97 The confinement well diameter has a strong influence on the motion pattern in the wells. In smaller
98 wells like 31 μm in diameter, even concentrated planktonic SM3 forms a single vortex (Fig. 2A),

99 whereas in larger wells, such as ones of 112 μm in diameter, swarming SM3 also breaks into
100 mesoscale vortices (Fig. 2B). The phase diagram shows a single swirl in small confinement for
101 both phenotypes of SM3. The patterns diverge as confinement size increases, but they converge
102 towards multiple swirls as confinement size reaches 144 μm and above (Fig. 2C). To further
103 compare the dynamics of the confined swarming and swimming SM3, the spatial correlation of
104 the velocity field was calculated for $d = 90 \mu\text{m}$ (where the motion patterns differ for swarming and
105 planktonic SM3) and for $d = 500 \mu\text{m}$ (where both motilities show mesoscale vortices) (see
106 Methods). We computed the correlation function for the inscribed square within a well, which
107 shows the extent to which the velocity at an arbitrary location correlated with the velocity at a
108 distance of Δr away from that location. In 90 μm wells, swarming SM3 velocity correlates
109 positively or negatively throughout the whole well (negative values have resulted from the
110 opposite sides of a single swirl). In contrast, the swimming velocity of planktonic cells of
111 comparable concentration does not correlate once $\Delta r > 25 \mu\text{m}$ (Fig. 2D). However, in a large open
112 space where both swarming and swimming SM3 break into small vortices, the correlation
113 functions look similar. The characteristic length as the curve first crosses $C_r(\Delta r) = 0$, which also
114 represents the size of the mesoscale vortices of planktonic and swarming SM3 is 27 μm and 33
115 μm , respectively (Fig. 2E).

116 We also tested other bacteria such as *Enterobacter* sp. SM1(Chen et al., 2020), *Serratia*
117 *marcescens* (including one lab strain Db10 and another strain, H3, isolated from a human
118 patient)(Chen et al., 2020), *Citrobacter koseri* (H6)(Chen et al., 2020), and *Bacillus subtilis*
119 3610(Chen et al., 2020). All the tested strains, with the exception of *B. subtilis*, showed similar
120 motion pattern divergence between confined planktonic cells and swarming cells like SM3 (Fig.
121 S1A, see discussion).

122 **The large single swirl behavior is indicative of cohesive cell-cell interaction**

123 We performed several experiments to explore parameters defining the divergence of motion
124 patterns in confinement. First, we rule out cell density difference as the reason for the difference
125 in the confined motion patterns by concentrating planktonic cells to a comparable density of a
126 naturally expanding swarm on agar (see Methods) before mounting the PDMS chip. Second, we
127 noticed that SM3 tends to get elongated when they swarm(Chen et al., 2020). We hypothesize that

128 elongated bacteria may enhance the local alignment of the rod-shaped cells and increase the
129 vortices' size in mesoscale turbulence (Doostmohammadi, Adamer, Thampi, & Yeomans, 2016).
130 Thus, we treated SM3 planktonic cells with cephalixin (CEP) which has been shown to elongate
131 *E. coli* (Hamby, Vig, Safonova, & Wolgemuth, 2018). This treatment indeed caused the cell length
132 of SM3 to reach that of swarming cells on average (Fig. 3A). However, we found no significant
133 change following the centrifugation and CEP treatment of the planktonic SM3 (Fig. 3B). Although
134 CEP treated planktonic SM3 has similar cell length, cell density, and cell speed as swarming SM3,
135 we could not restore the single swirl pattern in 74 μm confinement wells (Fig. 3C). Third, noticing
136 a surfactant rim on the swarming SM3 colony edge, we conjectured that surfactants secreted by
137 swarming SM3 might help align the swimmers in confinement. As a prototypical surface wetting
138 agent, surfactin was added in several concentrations to planktonic SM3 to test whether it could
139 promote a single-swirl motion pattern. However, it did not establish a stable single-swirl pattern.
140 Finally, we found that adding lyophilized swarming supernatant to swimming SM3 did not
141 increase the VOP either (Fig. 3C).

142 Unable to make the concentrated planktonic SM3 form a single swirl in the 74 μm well, we tackled
143 the problem from another angle, by altering the conditions of swarming SM3 in order to break the
144 single swirls. Initially, we tried to physically “disrupt” the swarming colony by rubbing the
145 swarming colony gently with a piece of PDMS offcut. This operation did not break the single swirl
146 pattern in the wells (Fig. 3D). Then, 0.2% D-mannose was added to the swarming colony to de-
147 cluster bacteria bundles due to cells' sticking to each other (Hamby et al., 2018). However, this
148 treatment could not alter the single swirl pattern, either (Fig. 3D). Finally, we diluted the swarming
149 cells in Lysogenic Broth (LB) by 20-fold. After re-concentrating the cells by centrifugation and
150 removing extra LB to recover the initial cell density, these “swarming” SM3 cells were pipetted
151 back on the agar plate. After this treatment, the previous single swirl turned to multiple swirls
152 under the confinement (Fig. 3D), suggesting that these cells now behave much like planktonic
153 cells. We conclude that the single swirl pattern depends on cohesive cell-cell interaction mediated
154 by biochemical factor/s removable by matrix dilution.

155 **Diluted swarming SM3 show unique dynamic clustering patterns**

156 We suspected that specific interactions between the neighboring swarming cells were weakened
157 or diminished upon dilution with the LB medium. A fifty (50) μL water droplet was applied to the
158 swarming and the concentrated planktonic SM3 colony edges to investigate the potential
159 intercellular alignment at a microscopic scale within the bacterial colony. In the diluted swarming
160 colony, groups of cells formed bacterial rafts, a characteristic feature previously associated with
161 gliding motility (Be'er & Ariel, 2019; Kearns, 2010a). Those cells within a polar cluster are moving
162 in the same direction in a cohesive pack at the same speed (Movie S3). In contrast, upon dilution
163 of the concentrated planktonic SM3, the cells disperse uniformly, and their moving directions
164 appear random (Movie S4). Swarming SM3 cells tend to move together near the agar surface,
165 while planktonic SM3 cells swim freely in the bulk fluid (Fig. 4A-B). We used the MATLAB PIV
166 toolkit to track the moving bacteria in the image sequences of diluted swarming and planktonic
167 SM3 for comparison. We found that swarming SM3 formed clusters with more than 20 cells on
168 average, while we did not see such clusters of planktonic SM3 cells (Fig. 4C-D). The lingering
169 clusters of cells in the swarming phase upon dilution point to a more substantial cell-cell cohesive
170 interaction than between planktonic cells.

171 **Numerical simulation reveals cell-cell interaction to be the key player**

172 To further verify that rafting in swarming is a crucially relevant factor to the motion pattern
173 discrepancy, we performed computer simulations using a zonal model for pair-wise interactions.
174 The interactions among the moving particles (short-range repulsion, velocity alignment, and anti-
175 alignment) are considered, all as functions of the particle-particle distance (Grossmann,
176 Romanczuk, Bar, & Schimansky-Geier, 2014, 2015). The particles' speed is fixed for simplicity,
177 but the initial particle positions and initial moving directions are randomized. In the simulations,
178 we interpret the rafting as due to a lower repulsion force and more substantial alignment among
179 the swimmers (described in Methods and Supporting Text). We simulated the situation of confined
180 swimmers and planktonic cells in different sizes of circular confinement, as performed in the
181 experiments. The simulation results mirror the experimental results. Both swimmers and planktonic
182 cells start with a single-swirl pattern; as the circle size is increased, the planktonic cells break into
183 a multi-swirl motion pattern earlier than the swimmers and finally both converge to the multi-swirl
184 region (Fig. 5A, compared with Fig. 2C; also see Fig. S3 and Movie S5). We then performed the
185 “dilution” simulation for both states, finding that swarming cells form dynamic polar clusters when

186 the cell density is around $\rho = 235$. In contrast, the planktonic cells form a “gas” phase without
187 clustering at all comparable densities (Fig. 5B, Movie S6). This result supports the experimental
188 observation in Fig. 4A. By encoding more vital cell-cell interaction among the swarming cells, our
189 computational model phenocopied the experimental results in both confinement and dilution
190 experiments.

191 **Identifying SM3 motility type on mice mucosal surface**

192 The difference in confined motion patterns enables us to detect bacterial swarming on surfaces
193 other than agar, including physiological environments such as mucosal. We are unaware of any
194 previous studies or examples regarding bacterial swarming on non-agar surfaces. There are
195 considerable technical challenges in dealing with uneven or more complex surfaces. The mouse
196 intestinal tissue, for instance, is more than 1 mm thick and non-transparent. Since light cannot
197 penetrate the tissue, observing bacteria directly on the tissue surface is not feasible. Staining or
198 fluorescence labeling may alter the bacterial swarming motility (e.g. we found that SM3 becomes
199 non-swarming once GFP labeled). If the bacterial cells are labeled biochemically, the fluorescence
200 signal weakens when the cells reproduce. As an alternative strategy, using PDMS chips coated
201 with fluorescent beads and then mounted on SM3 inoculated C57BL6 mouse intestine tissue, we
202 detected swarming motility based on the “collective” swirling motion of the beads (see Methods,
203 Fig. 6, and Movie S7&8). This experiment on the mouse intestine tissue confirms that bacterial
204 swarming indeed occurs on a non-agar, physiologically relevant surface.

205 **Discussion**

206 We have shown the motion pattern differences between PDMS chip confined planktonic and
207 swarming *Enterobacter* sp. SM3 in the size range of $40 \mu\text{m} \leq d \leq 90 \mu\text{m}$. Compared with previous
208 work, our experimental setup has the advantage of ensuring stable and sustainable patterns. First,
209 PDMS material does not harm living bacteria cells and is permeable to oxygen (Turner, Zhang,
210 Darnton, & Berg, 2010), thus ensuring continued oxygen exposure required for swarming (Chen et
211 al., 2020). Second, we mounted the microchip on a soft agar containing over 97% water, which
212 automatically fills the wells via permeability and capillary flow. Finally, the LB agar also provides
213 the necessary nutrients to fuel the bacterial movement in the wells. Therefore, bacterial cells
214 confined in the microwells remain motile for hours, much longer than in droplets surrounded by

215 mineral oil(Hamby et al., 2018; Wioland et al., 2013) or in microfluidic chambers with glass
216 surfaces(Beppu et al., 2017; Wioland, Lushi, et al., 2016), where bacteria movement typically
217 lasted no more than 10 minutes.

218 Prior studies have proposed different models to explain the circularly confined motion of rod-
219 shaped swimmers(Hamby et al., 2018; Lushi et al., 2014; Tsang & Kanso, 2015). However,
220 previous models cannot explain the motion pattern difference we observed for confined swarming
221 and planktonic SM3. Noticing that swarming SM3 washed in LB lost the single swirl pattern, we
222 hypothesize that other than cell length or cell speed, the strong cell-cell interaction may be a key
223 factor responsible for the persistence of single swirls in the wells. The mechanism of the rafting
224 phenomenon of swarming cells has not been fully deciphered yet(Kearns, 2010a). It might be due
225 to cohesive interaction among neighboring cells and hydrodynamic effects among 2D-confined
226 peritrichous flagellated bacteria(Li, Zhai, Sanchez, Kearns, & Wu, 2017). The cell-cell interaction
227 may further result from biochemical change of cell envelope during swarming (e.g., more long
228 sidechain lipopolysaccharides) or secretions(Armitage, Smith, & Rowbury, 1979). Once these
229 surrounding matrix or polymers are washed away by ~ 100-fold dilution, the cohesive interactions
230 are diminished, resulting in a loss of dynamic clusters in the dilution experiment, and multi-swirl
231 motion pattern under confinement. We confirm that lower repulsion and higher alignment are the
232 key factors that differentiate swimmers and planktonic cells by reproducing the experimental
233 results via numerical simulation. Future work is called upon to explore the swarmer rafting
234 phenomenon further and investigate the molecular basis for cell-cell cohesive interaction among
235 the swarming cells.

236 A spectrum of swarming bacteria manifests the same characteristic as SM3 (Fig. S1A). The
237 bacteria tested, including SM1, H6, H3, and Db10, all behave like SM3. They all showed clustering
238 or cohesive cell-cell interaction when the swarming colony was diluted and uniformly dispersed
239 when the concentrated planktonic cells were diluted. One notable exception is *Bacillus subtilis*.
240 Swarming and concentrated planktonic *Bacillus subtilis* 3610 show the same motion pattern across
241 different confinement sizes. For well diameter $d \leq 90 \mu\text{m}$, both swarming and swimming *B. subtilis*
242 form single swirls while for well diameter $d \geq 112 \mu\text{m}$, they both break into mesoscale vortices. *B.*
243 *subtilis* is a Gram-positive bacterium, different from SM3, SM1, H6, H3, and Db10. We speculate
244 that swarming *B. subtilis* does not have as strong a cell-cell interaction as SM3 and its gram

245 negative cohort we tested. The interaction is not so different between the swarming and planktonic
246 *B. subtilis* 3610 cells since we found the diluted swarming cells to disperse uniformly, and with
247 no clustering behavior, much like diluted planktonic cells. The swarming colony thickness for *B.*
248 *subtilis* may also play a role in defining the differences between this bacterium and the other strains.
249 It is known that swarming *B. subtilis* produces abundant surfactant, resulting in a wide-spread,
250 monolayer, non-compact colony(Be'er & Ariel, 2019; Jeckel et al., 2019). In contrast, swarming
251 SM3 and the other tested bacteria are multilayer colonies that can be as thick as 20 - 40 μm . The
252 thickness of SM3 swarm and that of its gram-negative cohort on agar may extend the strong cell-
253 cell alignment through the entire depth of PDMS wells, which is lacking among planktonic cells
254 of comparable concentration (Fig. S1B).

255 Our experiments on SM3 confirm the prediction made by Beppu *et al.* that single vortex occurs
256 when the confinement diameter d is smaller than a critical length l^* (Beppu et al., 2017). Here, the
257 critical length for swarming SM3 is $\sim 49 \mu\text{m}$, whereas, for concentrated planktonic SM3, it is ~ 17
258 μm . Interestingly, the same bacterial strain in different motility states has two distinct critical
259 lengths. Thus, we were able to use this property to identify the motility types on mouse mucosal
260 surfaces. The beads' motion is not a perfect swirl in every well on the colitic tissue because the
261 mucosal surface is not as smooth as the agar surface. There are sags and crests on the inflamed
262 mucosal surface due to the disrupted mucin layer(Chen et al., 2020). We conjectured that this
263 unevenness hindered the swirl formation to a certain extent. Indeed, intact swirl patterns were
264 spotted only on limited locations where the mucosal surface was relatively flat. Nevertheless,
265 capturing only a few wells where beads showed single swirl motion was sufficient to show that
266 swarming occurred on a mucosal surface.

267 Evidence of genetic and epigenetic regulation(Daniels, Vanderleyden, & Michiels, 2004;
268 Morgenstein, Szostek, & Rather, 2010; Tremblay & Deziel, 2010; Wang, Frye, McClelland, &
269 Harshey, 2004), and cell morphology changes (e.g., cell elongation and hyper-flagellation),
270 indicates that swarming is a different phenotype from swimming. Lacking comparison under the
271 same experimental conditions, one might suspect that bacterial swarming might be a dense group
272 of cells swimming on a surface(Kearns, 2010a). Here, through geometry confinement, we show
273 that *Enterobacter* sp. SM3 swarming manifests different biophysical characteristics from
274 swimming. This study's key experimental method differentiates swarming motility from

275 swimming motility at mesoscopic or even macroscopic scales, providing a visual assay to detect
276 swarming behavior on either an agar or tissue surface. This study's findings provide the rationale
277 for developing applications such as isolating bacterial swimmers from a polymicrobial environment
278 and developing diagnostics for the presence of *in vivo* swarming (e.g., detecting urinary or fecal
279 swarming bacteria in catheter infections or intestinal inflammation, respectively)(Arikawa &
280 Nishikawa, 2010; Lane et al., 2007). Additionally, the sensitivity to confinement size indicates that
281 a quantitative ranking system for different swimmers could be established based on the
282 characteristic well size that stabilizes the confined motion pattern into a single swirl. Such a
283 ranking system will be significant for future investigations on the implications of swarming
284 bacteria in host physiology and pathophysiology.

285 **Methods**

286 **PDMS confinement sheet fabrication.** Polydimethylsiloxane (PDMS) microwell confinement
287 sheets with different combinations of well sizes and depths were fabricated using a soft
288 photolithography technique. Patterns of the confinement were first designed using the software
289 "L-Edit" and then uploaded into a maskless aligner (MLA 150, Heidelberg). On a 3.5-inch silicon
290 wafer (University Wafer Inc.), photoresist gel SQ25 (KemLab, Inc.) was spin-coated at 2,000 rpm
291 (spin speed varies according to the desired coating thickness). After baking, UV exposure, and
292 chemical development, the microwells' designed pattern was shown on the wafer (molding). Then,
293 PDMS (Dow Corning Sylgard 184) base elastomer was mixed with the curing agent at the ratio of
294 10:1 in weight. The mixture was cast onto the patterned silicon wafer. Two grams of the mixture
295 ended up with a PDMS sheet about 0.5 mm thick. The PDMS solidified at room temperature within
296 48 hours and it was cut into pieces and peeled off from the silicon wafer before use (demolding).

297 **Bacterial growth and confinement (Fig. 7A).** *Enterobacter* sp. SM3 is a novel swarming
298 bacterial strain isolated from inflammatory mice(Chen et al., 2020). SM3 was transferred from -
299 80°C glycerol stock to fresh LB (Lysogeny Broth: water solution with 10 g/L tryptone, 5 g/L yeast,
300 and 5 g/L NaCl) and shaken overnight (~ 16 h) in a 37°C incubator at 200 rpm. For swarming
301 under confinement assay (Fig. 7A, red arrows), two (2) µL overnight bacterial culture was
302 inoculated on the center of an LB agar plate (10 g/L tryptone, 5 g/L yeast, 5 g/L NaCl, and 5 g/L
303 Agar; volume = 20 mL/plate) and kept in a 37°C incubator. After 2.5 h of swarming, a PDMS chip

304 (~ 1 cm²) was mounted upon the edge of the swarming colony and the Petri dish was transferred
305 onto the microscope stage for observation. For swimming under confinement assay (Fig. 7A, blue
306 arrows), overnight bacterial culture was resuspended in fresh LB (1:100 in volume) and shaken in
307 the 37°C incubator at 200 rpm for 2.5 h. The freshly grown culture was centrifuged at 1,500 g for
308 10 min and ~ 98.6% of the supernatant was removed so that the resultant cell density is about 70
309 times the freshly grown culture. Ten (10) µL concentrated bacteria culture was inoculated on the
310 LB agar plate, and the PDMS chip was mounted immediately. The plate was then transferred onto
311 the microscope stage for observation. For other bacteria strains, including *Bacillus Subtilis* 3610,
312 the procedure was the same as that of SM3. There are thousands of wells on one PDMS chip, and
313 when mounted on a bacteria spot or colony edge, hundreds of them are occupied by bacteria. The
314 PDMS chip was first brought to contact with the bacteria and then gently mounted onto the agar.
315 By doing so, there was a cell density gradient across an array of wells, with the wells closer to the
316 bacteria spot or colony center having relatively higher cell density. We focused on the area where
317 the confined bacteria showed collective motion, i.e. the cell density was not too high to
318 oversaturate the well, or too low so that each cell was moving independently.

319 **Bacterial cell density measurement (Fig. 7B).** Two and half hour (2.5 h) freshly grown SM3 was
320 subjected to different factors of dilution in LB, such as 10², 10³, until 10⁸. Fifty (50) µL of each
321 diluted culture was inoculated and spread on 1.5 % LB agar plate (10 g/L tryptone, 5 g/L yeast, 5
322 g/L NaCl, and 15 g/L Agar; volume = 20 mL/plate) and was incubated at 37°C for 16 h. Bacterial
323 colonies appeared on the agar plates and the number of colonies was counted for the dilution that
324 resulted in the colony's number on the order of 100. The colony forming unit per microliter
325 (CFU/mL) was calculated by dividing the colony number by the sampled volume. For swarming
326 SM3, the cell density was measured similarly. On the edge of the swarming colony, a chunk of
327 swarming SM3 (~ 1 mm wide) was picked by an eight (8) mm-wide square spatulate containing a
328 small agar bottom to ensure all the cells in that region were sampled. The 1 mm x 8 mm chunk of
329 swarming SM3 was then mixed into 1 mL LB for CFU determination. The colony thickness was
330 assumed to be uniform across the sample. It was measured by microscopy focusing on the top of
331 the colony and the top of the agar surface (i.e., at the bottom of the colony), keeping track of the
332 fine adjustment knob readings. Particles of baby powder (~ several micrometers in diameter) were
333 spread on the swarm colony surface and the agar to aid in the microscope focus. The thickness of
334 the swarming colony was calculated based on the calibration of the knob turning tick readings.

335 Then the cell density was estimated by CFU/mL. CFU was calculated for both swarming and
336 swimming SM3 to ensure the cell densities of these two cases were comparable inside the wells.
337 We consider colony-forming unit counting a better way to control the live cell number than merely
338 using the volume fraction because 1), Dead cells that count in the volume fraction will not
339 contribute to the motion in the well, but they will be excluded in CFU calculation; 2), It is
340 challenging to measure the volume of dense bacterial suspension using pipetting method due to
341 high viscosity.

342 **Bacterial cell length and motility.** For swimming SM3, 2.5 h freshly grown culture was diluted
343 100 times in LB and 50 μ L of which was transferred on a glass slide and covered with a coverslip.
344 The sample slide was placed under the microscope (Olympus CKX41, 20X), and image sequences
345 were captured. Cell lengths were measured using ImageJ (v1.59e) freehand label tool. Cell speed
346 was calculated as the traveling trajectory length divided by the traveling duration (\sim 1s). For
347 swarming SM3, a chunk of swarming bacteria was collected from the swarming colony edge and
348 mixed with 1 mL LB. A glass slide and a cover slip sandwiched a droplet of 50 μ L mixed culture,
349 and the rest of the procedure was the same as that for the swimming SM3.

350 **Swimming SM3 with different treatments. i), Cephalexin treatment.** Overnight SM3 culture
351 was diluted 100 times in fresh LB and incubated in a 37°C shaker at 200 rpm for 1.5 h. Cephalexin
352 (CEP) (C4895; Sigma-Aldrich) was added to the culture so that the CEP's resultant concentration
353 was 60 μ g/mL. The culture was kept in the shaker for another two (2) h before use. **ii), Surfactin**
354 **additions.** After 2.5 h regrown culture was centrifuged, more supernatant was removed than usual,
355 and surfactin (S3523; Sigma-Aldrich) was added so that the resulting concentrations of surfactin
356 were 10, 50, 100, 500 μ M. At the same time, the cell density remained comparable to that of
357 swarming SM3. **iii), Addition of swarming supernatant.** Before swarming SM3 covered the plate,
358 the colony was scratched carefully using a PDMS (\sim 0.5 cm²) and transferred into 1 mL deionized
359 water. The mixture was sucked into a syringe and filtered with a 0.2 μ m filter. The solution was
360 then lyophilized to powder and then dissolved into the concentrated planktonic SM3 of roughly
361 the same volume as the collected swarm fluid. Thus, the concentration of the swarming supernatant
362 was kept the same to subject the concentrated planktonic SM3 to.

363 **Swarming SM3 with different treatments. i), Soft scratching with PDMS.** After SM3 swarmed
364 on the agar plate for 2.5 h, a piece of PDMS (~ 0.5 cm²) was used to softly scratch the edge of the
365 swarming colony so that the swarming cells were disturbed. A PDMS confinement chip was then
366 mounted on the disturbed region for observation. **ii), Spun down in LB.** After swarming for 2.5
367 h, SM3 cells were collected from the colony's edge using the blotting method(N. Darnton, Turner,
368 Breuer, & Berg, 2004). The cells were blotted by a piece of spare PDMS and transferred to 1 mL
369 LB. The swarming cells were centrifuged at 1,500g, and LB was removed to restore the initially
370 high cell density. Ten (10) µL of the swarming cells thus treated were inoculated on a new swarm
371 agar and a PDMS confinement chip was mounted for observation. **iii), D-mannose.** A droplet of
372 50 µL 0.2% (w/v) D-mannose (Cas No. 3458-28-4; RPI) was pipetted on a swarming SM3 colony
373 edge. After 1-2 minutes, when the cell density became uniform again, a piece of PDMS
374 confinement chip was applied to the D-mannose treated region for observation under the
375 microscope.

376 **VOP measurement and spatial autocorrelation function.** Image sequences of swarming or
377 swimming SM3 under confinement were taken by a microscope camera (ThorLabs, Kiralux
378 CS505MU) and then processed using a particle image velocimetry (PIV) package in MATLAB.
379 The velocity field was marked for the confined bacteria and the VOP was calculated using the
380 equation in Fig. 1E. Using the velocity field information, we calculated the spatial autocorrelation
381 function through the equation $C_r(\Delta r) = \langle \frac{v(r_0) \cdot v(r_0 + \Delta r)}{|v(r_0)|^2} \rangle$, where r_0 is the local position vector
382 and Δr is the displacement vector(Patteson, Gopinath, & Arratia, 2018). A Python script was
383 written to calculate all the C_r values in the region of interest (ROI) with a label of Δr values. These
384 C_r values were then plotted as a function of Δr .

385 **Clustering analysis.** On the swarming SM3 colony edge or concentrated swimming SM3
386 inoculation, a droplet of 50 µL deionized water was added via a pipette. Once the fluid flow
387 stabilizes, image sequences were captured at the diluted swarming or planktonic SM3 sample
388 locations. In a region of 130 µm x 130 µm, the velocity field was calculated using the PIV toolkit,
389 and the vectors with magnitude below four (4) µm/s were removed. The purpose of the vector
390 validation was to exclude non-motile bacteria. Once the moving cells were identified, a Python
391 script was implemented to perform the clustering analysis using the function of DBSCAN(Ester,
392 1996) where the parameter ϵ was set to 50, which specifies how close points should be to each

393 other to be considered a part of a cluster, and the minimum number of points to form a cluster was
394 set to 20.

395 **Numerical Simulations.** The numerical simulation consists of a 2D system of N particles. The
396 position \mathbf{r} of each particle is modeled via the following overdamped Langevin equation:

$$397 \quad \partial_t \mathbf{r}_i = v_0 \hat{p}_i - \sum_{j \neq i} G_\theta(d_{ex}, r_{ji}) + \sqrt{2D_T} \xi_i \quad (1)$$

398 It is assumed that particles are cruising at a constant speed of v_0 in the direction of $\hat{p}_i =$
399 $[\cos(\theta_i), \sin(\theta_i)]$. The second term includes the exclusion forcing term from all neighboring
400 particles residing at a distance r_{ji} closer than the exclusion range d_{ex} . The last term is the thermal
401 fluctuation term with the translational diffusivity D_T and a zero-mean and delta-correlated noise
402 term ξ . The direction of motion θ_i of each particle is updated by the interaction terms F_θ , which
403 includes alignment, anti-alignment and repulsion effects with all neighboring particles and the
404 rotational diffusion term with diffusivity of D_r and noise term ζ :

$$405 \quad \partial_t \theta_i = \sum_{j \neq i} F_\theta(\mathbf{r}_{ji}, \hat{p}_i, \hat{p}_j) + \sqrt{2D_r} \zeta_i \quad (2)$$

406 The details of the binary interaction terms G_θ and F_θ are provided in the Supporting Information.
407 The simulation starts with random initial position and orientations, followed by numerical
408 integration of equations (1) and (2) using a first-order Euler method. The integration time step Δt
409 is chosen small enough to ensure numerical stability and independence of long-term dynamics
410 from the time step increment. The interaction of particles with a circular bounded domain is
411 modeled through a reflective boundary condition. The particles are reflected off the boundary with
412 an angle equal to their incident angle. In all diluted cases, reflecting solid boundary is replaced
413 with a periodic boundary condition to ensure that boundary scattering does not affect the dynamics
414 in bulk.

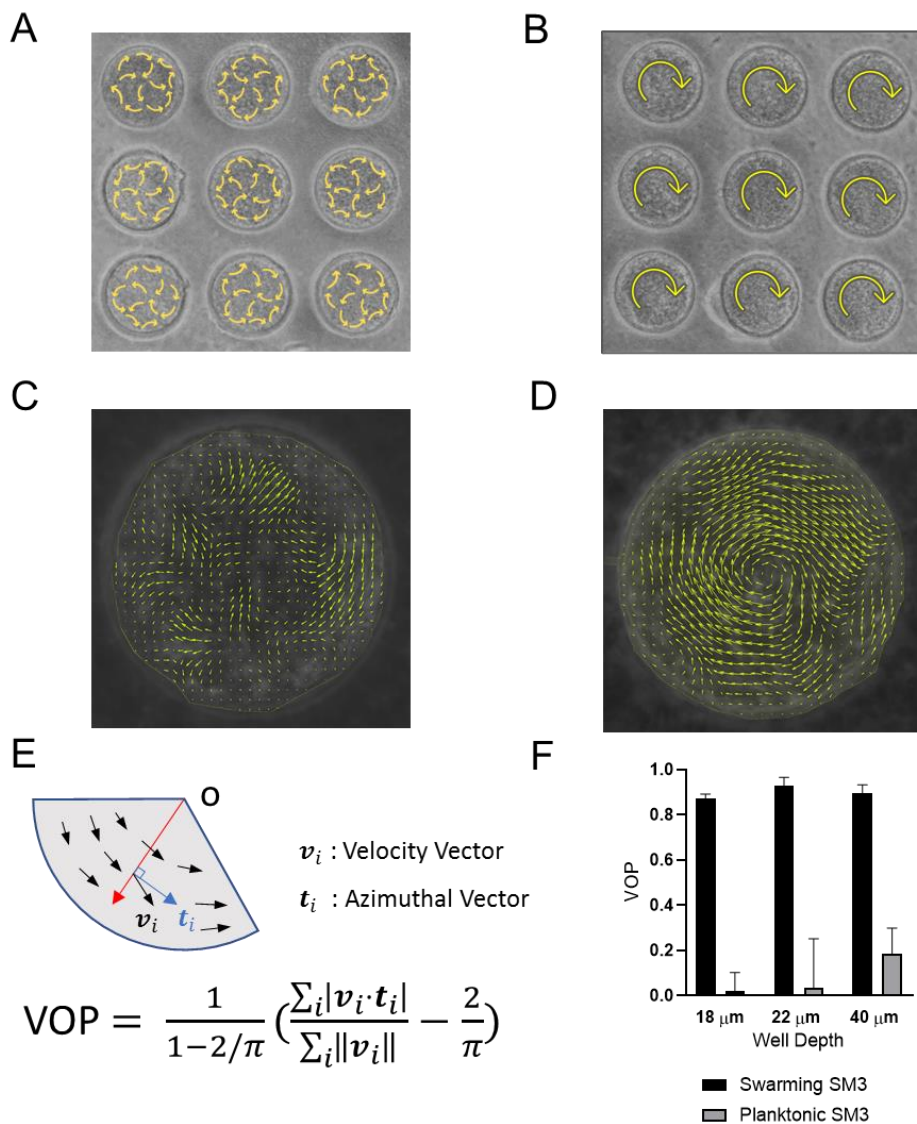
415 **Detecting bacterial motility on mouse intestinal mucosal tissue using PDMS chips.** Six-week-
416 old female C57BL/6 mice (Jackson Laboratories, Bar Harbor, ME; #000664) were administered
417 3%(w/v) DSS (Dextran Sulfate Sodium) (MPI; # 160110) in animal facility drinking water daily
418 to induce acute colitis(Chen et al., 2020). After 9-12 days, when the mice's weight loss reached
419 20%, mice were euthanized using isoflurane anesthesia and large intestines were harvested. For
420 controls, conventional six-week-old female C57BL/6 mice exposed to drinking water with DSS-

421 vehicle added were also sacrificed and the intestines were collected. This study was approved by
422 the Institute of Animal Studies at the Albert Einstein College of Medicine, Inc (IACUC #
423 20160706 & 00001172). Intestinal tissue was surgically exposed, cleansed with 35%(v/v) ethanol,
424 and rinsed with PBS twice. The mucosal surface of the tissue was cultured (on gar streaks) for any
425 residual bacteria and only used when there were no bacterial colonies on aerobic or anaerobic
426 culture. Prior to experiments, a portion of the mucosal tissue was also harvested after ethanol
427 cleansing for histology and to validate its histologic integrity with respect to non-cleansed DSS-
428 exposed tissue. Tissues were spread on a 1% agar plate with the inner side facing up, and overnight
429 SM3 bacterial culture was inoculated on one end of the tissue. The agar plate was incubated under
430 37°C for 4.5 hours to allow SM3 bacteria to duplicate and move on the tissue surface. PDMS chips
431 (d = 38 μ m) were coated with 0.5 μ m fluorescent beads (Dragon green; Bangs Laboratory, IN)
432 and cut into strips to fit the tissue's size. The PDMS strip was mounted on and covered the tissue
433 surface. Bead motion was observed under the fluorescence microscope (Olympus CKX41) with
434 20X objectives.

435 **Acknowledgment**

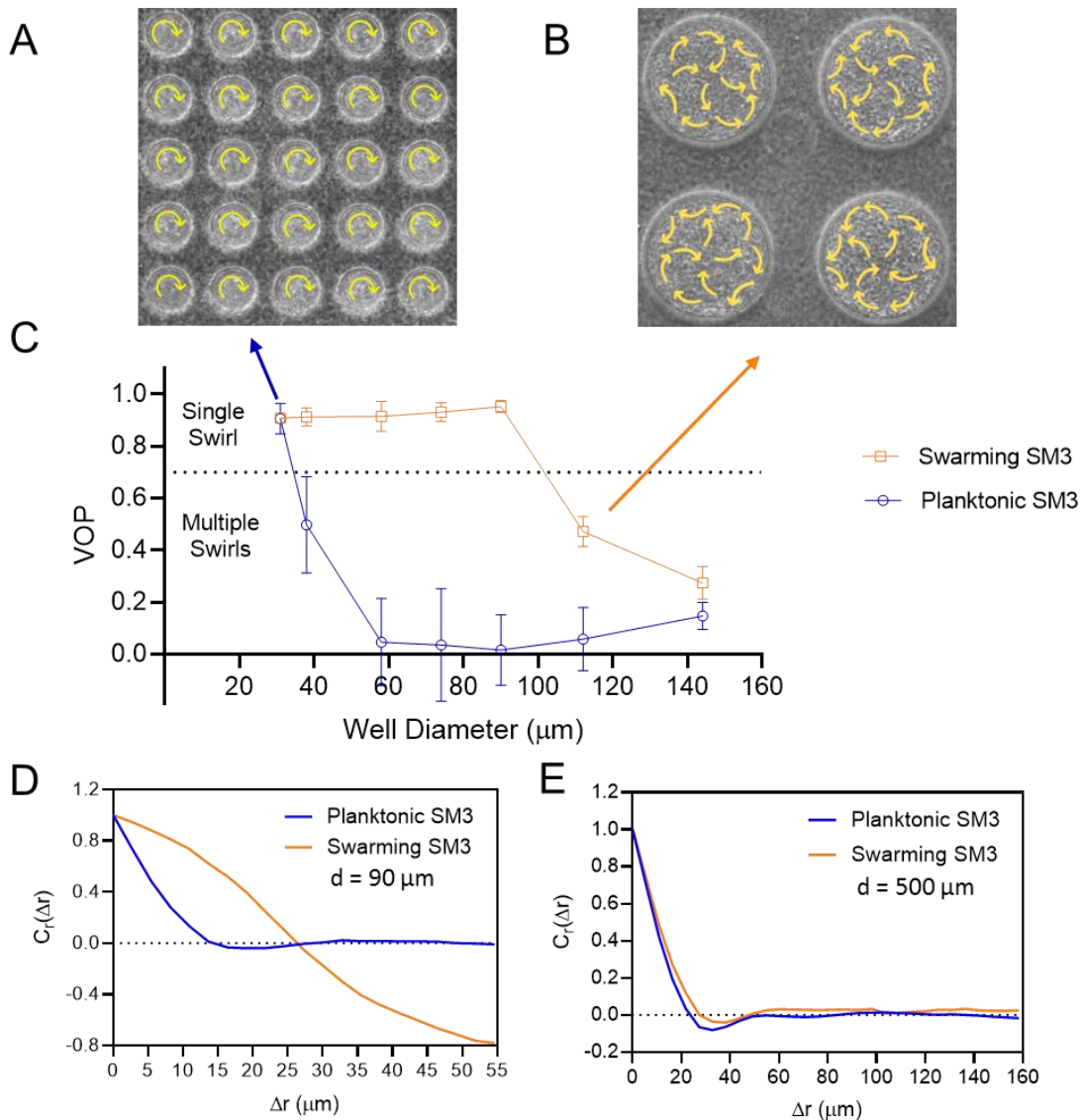
436 We thank Daniel B. Kearns from Indiana University at Bloomington for providing the *Bacillus*
437 *subtilis* 3610 bacteria strain, Cori Bargmann at Rockefeller University, for gifting us the bacteria
438 strain *Serratia marcescens* Db10. We thank Hui Ma for his assistance in the cleanroom and
439 discussion of the work. N.I.H. Grant 1R01CA222469-01 supported this work.

440 **Figure 1**



441
 442 **Figure 1 | Swirls of *Enterobacter* sp. SM3 under circular confinement.** (A-B) Motion pattern of
 443 concentrated planktonic (A) and swarming (B) SM3 in the PDMS microwells of 74 μm in diameter. Circular
 444 arrows indicate the direction of bacterial collective motion. (C-D) Velocity field of concentrated planktonic
 445 (C) and swarming (D) SM3 in a single microwell. (E) Illustration of how vortex order parameter (VOP) is
 446 defined. $|\cdot|$ denotes the absolute value while $\|\cdot\|$ denotes the Euclidean norm. (F) VOP of swarming and
 447 swimming SM3 in 74 μm microwells of 3 different depths. The sample size $n = 5$ for each group and data
 448 are represented as mean and standard deviation (\pm SD).

449 **Figure 2**



450

451

452 **Figure 2 | The effect of well diameter on confined *Enterobacter Sp.* SM3 motility patterns. (A-B)**

453 Motion pattern of concentrated planktonic SM3 confined in 31 μm (A) and swarming SM3 confined in 112

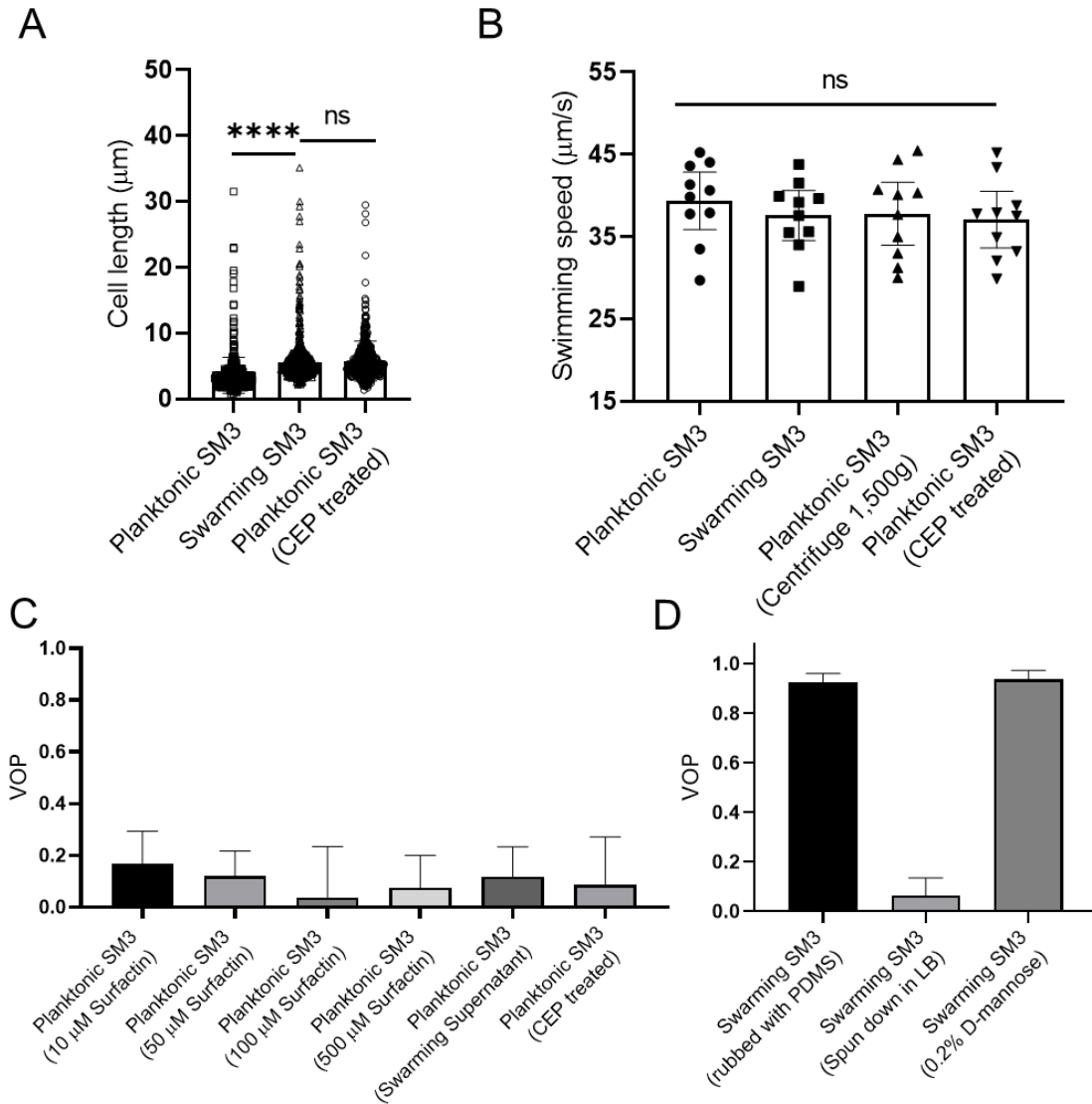
454 μm (B) diameter microwells. (C) VOP of swarming and concentrated planktonic SM3 as a function of well

455 diameter. The error bars represent the standard deviations (\pm SD) for each data point, and the sample size

456 is $n = 5$. (D-E) Spatial autocorrelations of the bacterial velocity field in the well diameter of 90 μm (D) and

457 500 μm (E). Unless otherwise noted, the depth of the wells is 22 μm.

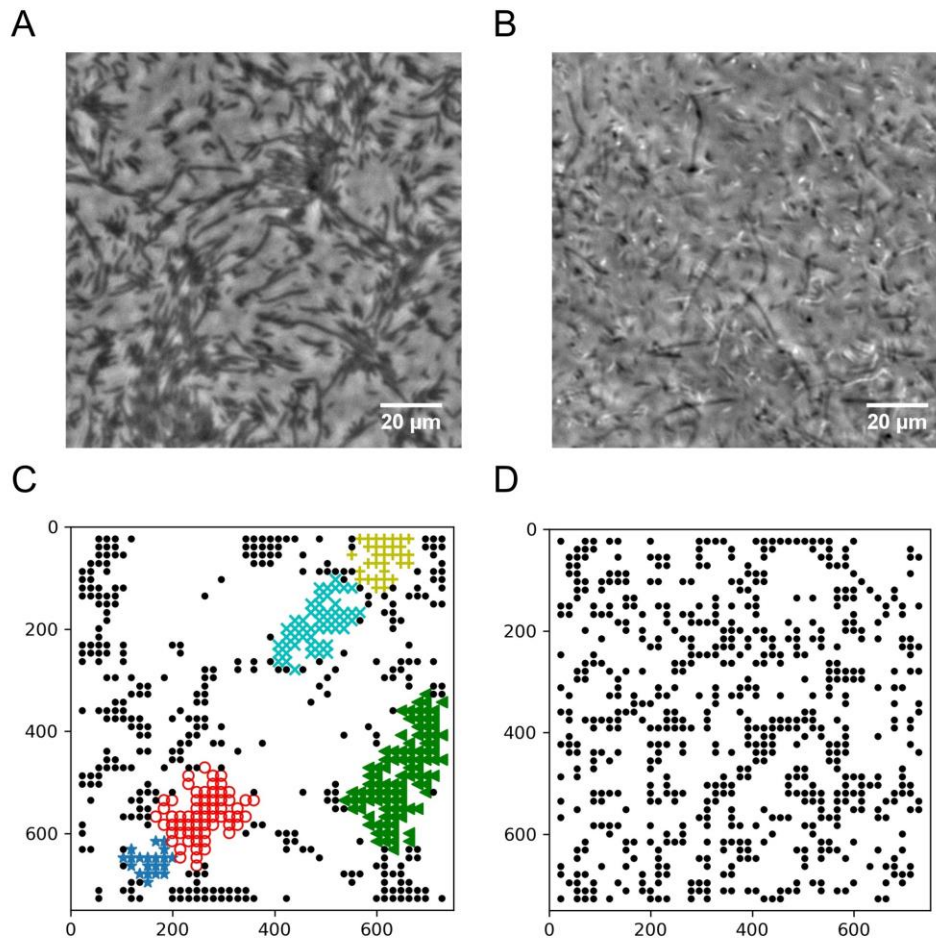
458 **Figure 3**



459

460 **Figure 3 | Factors that possibly influence the bacterial motion pattern in the well.** (A) Bacterial cell
 461 length of planktonic, swarming, and cephalixin (CEP) treated planktonic SM3, n = 500 for each group.
 462 Data are represented as median and interquartile range. **** indicates $P < 0.0001$. ns indicates not
 463 significant (Kruskal-Wallis test). (B) Bacterial cell speed of swimming, swarming, centrifuged, and CEP
 464 treated swimming SM3, n = 10 for each group. ns, not significant, one-way ANOVA followed by Tukey's
 465 post hoc test. (C) VOP of swimming SM3 under 74 μm diameter confinement with different treatments, n
 466 = 5 for each group. (D) VOP of swarming SM3 under 74 μm diameter confinement with different treatments,
 467 n = 5 for each group. B-D, Data are represented as mean and standard deviation (\pm SD).

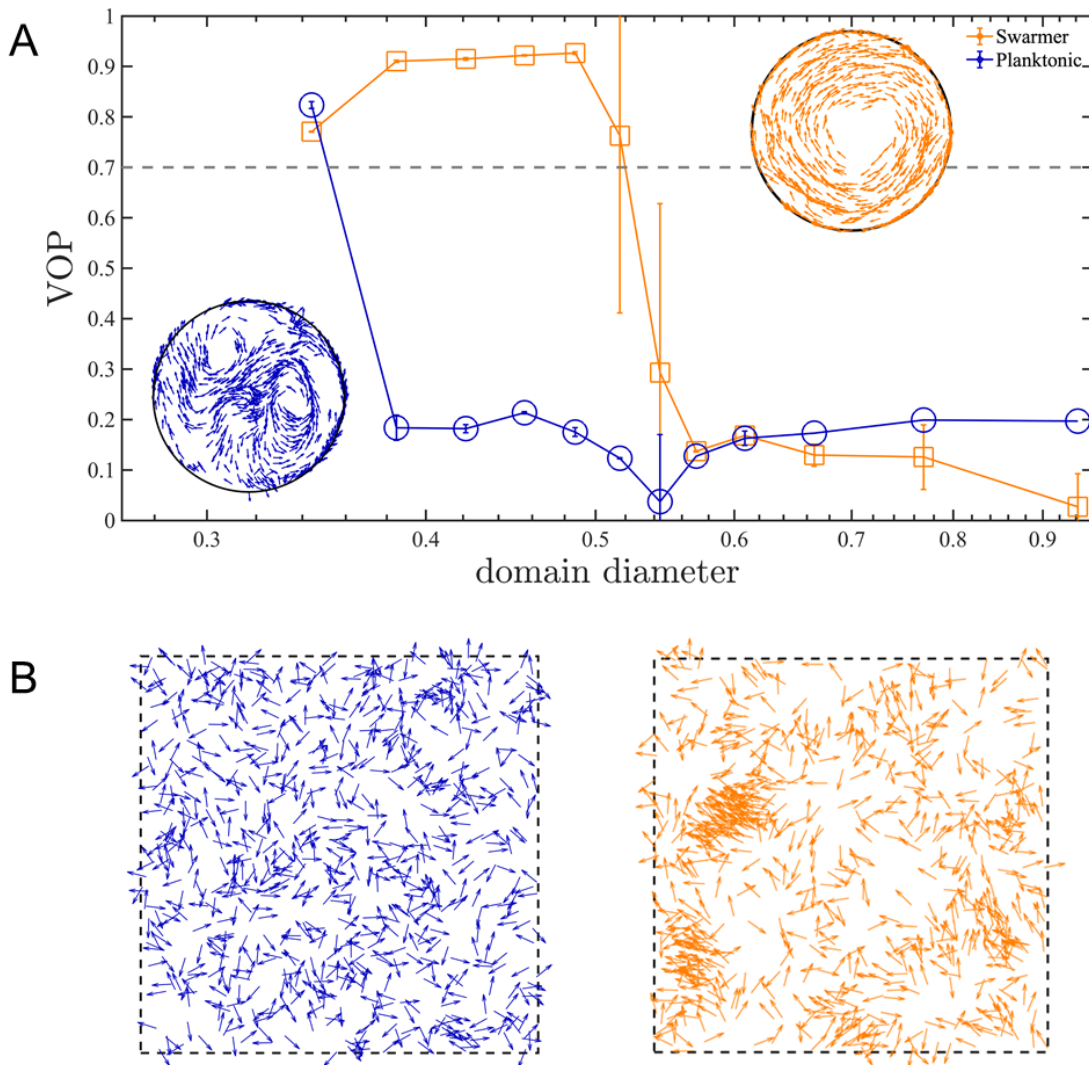
468 **Figure 4**



469

470 **Figure 4 | Spatial distribution of swarming and swimming SM3 cells.** (A-B) Snapshots showing diluted
471 swarming SM3 (A) and planktonic SM3 (B) on a soft agar surface, respectively. (C-D) DBSCAN clustering
472 analysis of diluted swarming SM3 (C) and planktonic SM3 (D). Black dots represent moving bacterial cells
473 and colored markers show cells in clusters, as determined by the program. The axis represents the dimension
474 of the image in pixels.

475 **Figure 5**



476

477 **Figure 5 | Numerical simulations of planktonic and swarming SM3 in confinement and open space.**

478 (A) VOP of swarming and concentrated planktonic SM3 as a function of well diameter. The error bars

479 represent the standard deviations (\pm SD) for each data point, and the sample size is $n = 5$. The circles on the

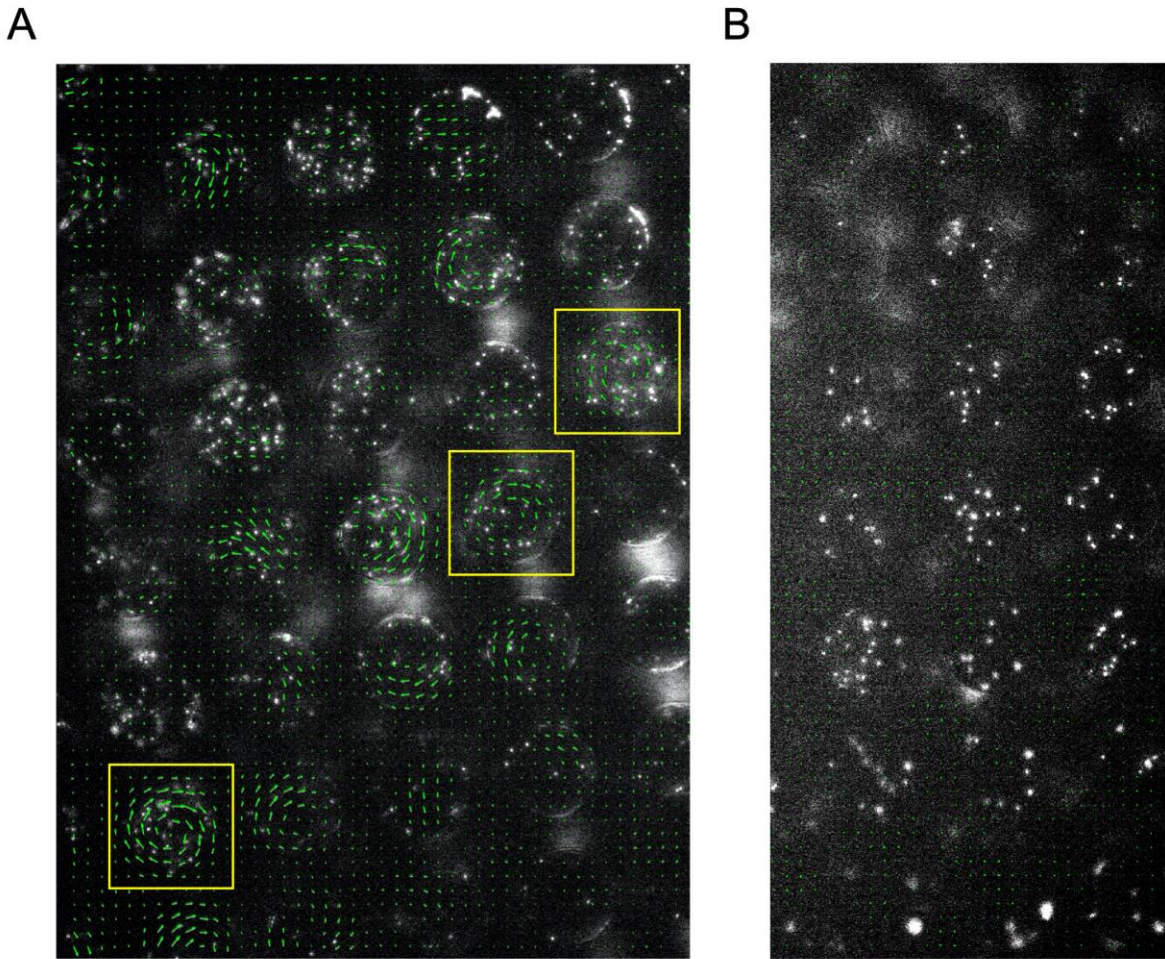
480 upper right corner and the lower left corner show representative motion patterns of swarmer and

481 concentrated planktonic cells in the confinement size between 0.38 and 0.5. (B) Planktonic cells (left) and

482 diluted swarming cells (right) with same cell density in a space of periodic boundary condition.

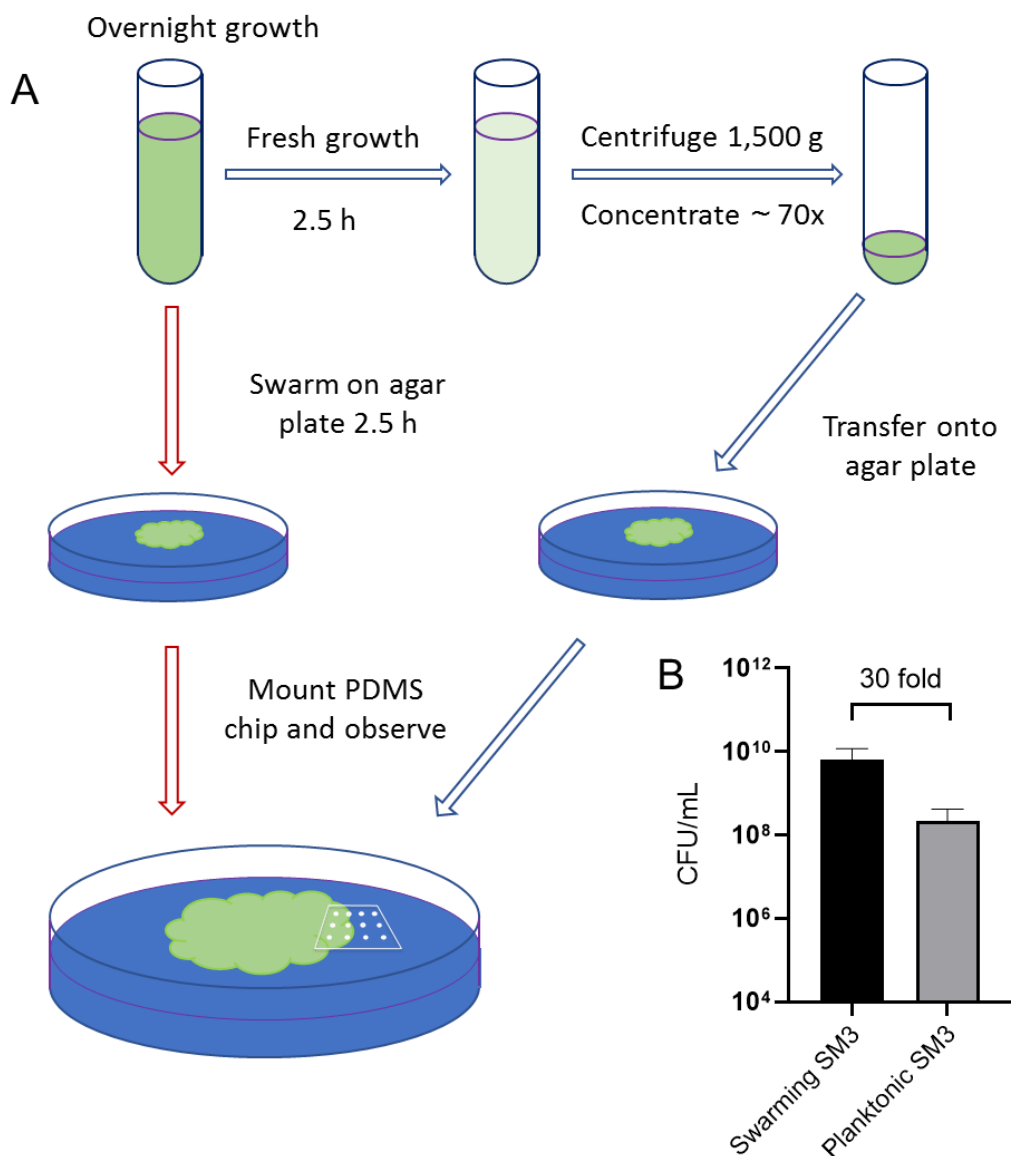
483

484 **Figure 6**



485
486 **Figure 6 | Motion of fluorescent beads in microwells mounted on infected murine tissue.** PDMS chips
487 were coated with 0.5 μm fluorescent beads and mounted on SM3 inoculated colitic (A) or non-colitic (B)
488 mice intestine tissue surfaces. The beads motion was measured after 4.5 hr incubation. Average velocity
489 field was calculated by tracing the beads motion using PIV toolkit. (A) On colitic tissue, wells with VOP >
490 0.7 were found and marked with yellow squares. We conclude that, in these wells, the single swirl motion
491 pattern of the beads was powered by the confined swarming SM3. Since the tissue surface was not as
492 smooth as on agar surface, the motion of the beads in some wells did not form a complete vortex, yet jets
493 indicating partial vortices were discernable. (B) On a normal tissue lacking swarming bacteria, the average
494 velocity of the beads in the wells due to random motion was close to zero, giving rise to uniformly small
495 VOP values. We could infer that the confined SM3 in these wells were predominantly swimming rather
496 than swarming.

497 **Figure 7**



498
499 **Figure 7 | Illustration of experimental procedure.** (A) Schematic of sample preparation procedure. Red
500 arrows represent the assay procedure for swarming bacteria. Blue arrows represent the assay procedure for
501 swimming planktonic bacteria. (B) Cell density measured by colony forming unit (CFU/mL) of swarming
502 SM3 and swimming SM3. Swarming SM3 cell density is measured after SM3 swarming on an agar surface
503 for 2.5 h while swimming SM3 cell density is measured for overnight SM3 culture being regrown in fresh
504 Lysogeny Broth (LB) for 2.5 h. Since cell density of swarming SM3 was higher than that of planktonic
505 SM3, the latter was concentrated before being applied on the agar plate to acquire comparable cell density.

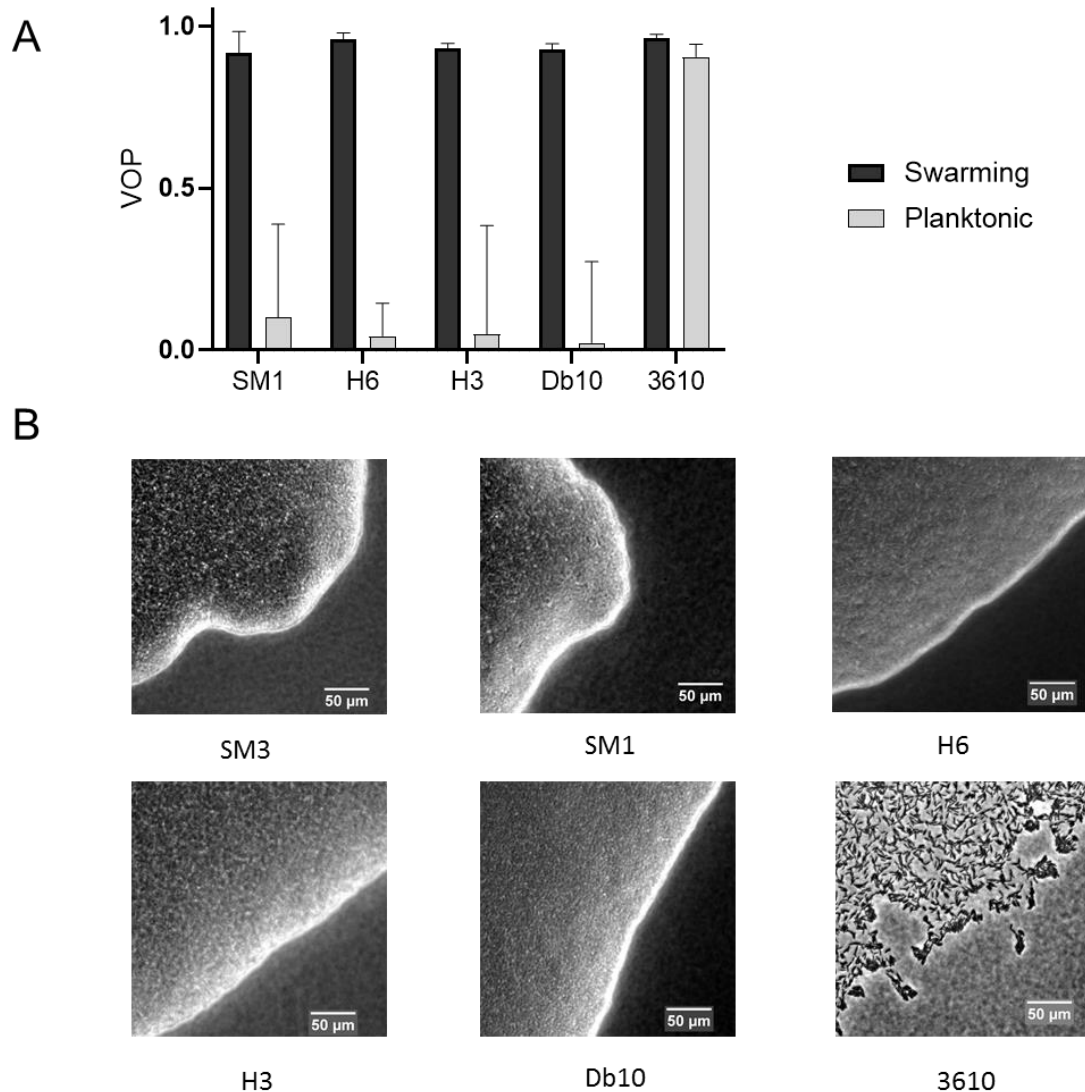
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- 607

609 **Supporting Information**

610 **Figure S1**



611

612

613 **Figure S1 | Comparison of Vortex Order Parameter (VOP) under confinement and swarm front**

614 **among several bacteria species.** (A) VOP of concentrated planktonic (SM1) and swarming (SM3)

615 *Enterobacter* sp., *Citrobacter koseri* (H6), *Serratia marcescens* (H3), *Serratia marcescens* (Db10),

616 and *Bacillus subtilis* 3610 confined in the PDMS microwells of 58 μm in diameter and 22 μm in depth. The

617 bars indicate averages with standard deviation (+SD) over five microwells. (B) Swarm in front of the tested

618 bacteria. *B. subtilis* 3610 forms a monolayer, loose swarming colony while all the other bacteria strains

619 form multilayer, compact swarming colonies.

620 **Supporting Text: Mathematical Modelling and Computer Simulation**

621 **A simplified treatment of swarming bacteria.**

622 Most particle-based models for self-propelled microswimmers incorporate detailed
623 hydrodynamics of elongated rods in a low Reynolds number environment (Costanzo, Di Leonardo,
624 Ruocco, & Angelani, 2012; Lushi & Peskin, 2013; Lushi, Wioland, & Goldstein, 2014; Saintillan
625 & Shelley, 2007). However, the dynamics of bacterial swarming comprise a complex interplay
626 between several physical and chemical interactions that go beyond hydrodynamic and steric effects.
627 Cell interactions with the extracellular polymeric network, mechanical locking, and intertwining
628 of flagella and formation of intercellular bundles between adjacent swimming cells (Copeland &
629 Weibel, 2009; Kearns, 2010) are a few examples whose underlying mechanisms are not fully
630 understood. In the absence of a comprehensive model that captures many interactions among
631 swarming bacteria, we seek a simplified description of active particles interacting via competing
632 interactions that capture the essential dynamics of both swarming and planktonic bacteria. Our
633 focused aim in connection with the experimental study in this report is to discern the distinct,
634 collective behaviors of swarming bacteria from their planktonic counterpart, in comparable
635 concentration, and under the extent of same spatial confinement.

636 There are numerous approaches for incorporating the relevant physical interactions between active
637 particles (Grossmann, Romanczuk, Bar, & Schimansky-Geier, 2014, 2015; Wensink et al., 2012;
638 Wensink & Lowen, 2012) (readers are referred to Bär *et al.* for a recent review (Bar, Grossmann,
639 Heidenreich, & Peruani, 2020), for example, on models for dry and wet interacting self-propelled
640 rods). Here, we choose the binary interaction model introduced by Großmann et al. (Grossmann et
641 al., 2014, 2015) based on the fact that hydrodynamic couplings among the swimmers can induce
642 both alignment and anti-alignment effects (Baskaran & Marchetti, 2009). The simplified model we
643 employ also allows us to implicitly embed the unknown interactions of cells with extracellular
644 polymeric network and possibly, mechanical locking of flagella between adjacent cells in
645 alignment, anti-alignment, and repulsion torque terms.

646 **Numerical model and simulation.**

647 The dynamics of N interacting active particles have been modeled in a 2-dimensional space using
 648 the overdamped Langevin-based equations, assuming that inertia is negligible in a low Reynolds
 649 number environment. The position \mathbf{r} and orientation θ of particle i are calculated using the
 650 following stochastic differential equations:

$$\partial_t \mathbf{r}_i = v_0 \hat{\mathbf{p}}_i - \sum_{j \neq i} k_{ex} r_{ji} \mathcal{H}(d_{ex} - r_{ji}) + \sqrt{2D_T} \xi_i \quad (1)$$

$$\partial_t \theta_i = \sum_{j \neq i} F_\theta(\mathbf{r}_{ji}, \hat{\mathbf{p}}_i, \hat{\mathbf{p}}_j) + \sqrt{2D_r} \zeta_i \quad (2)$$

651 In Eq. (1), the particles' self-propulsion speed is set to be a constant v_0 along the direction $\hat{\mathbf{p}}_i =$
 652 $[\cos(\theta_i), \sin(\theta_i)]$. This simple assumption is based on our experimental observations, suggesting
 653 that the bacterial velocity in the suspension is largely independent of the local cell density. The
 654 second term incorporates the central exclusion force term with a spring constant k_{ex} , which acts
 655 over the relative distance r_{ji} with all the neighboring particles j . This exclusion force term applies
 656 only when r_{ji} gets smaller than the exclusion range d_{ex} (represented as a Heaviside function \mathcal{H}).
 657 The last term in Eq. (1) is the Brownian fluctuation term with the corresponding translational
 658 diffusivity D_T and ξ_i is the white noise with zero mean and correlation $\delta(t)$.

659 Two terms influence the temporal change in the orientation of each particle. The first term on the
 660 right-hand side of Eq. (2) includes all the binary interaction terms. The last term on the right-hand
 661 side of Eq. (2) is the contribution from the angular Brownian fluctuation with the rotational
 662 diffusion D_r and a zero mean and delta-correlated stochastic white noise ζ . In the present study,
 663 we employ the pair-wise interaction model introduced by Grossman and co-workers (Grossmann
 664 et al., 2014, 2015), which successfully reproduces various macroscopic patterns that occur in dense
 665 bacterial suspensions. The pair-wise interaction term is based on a zonal model (illustrated in
 666 Figure S2 below), capturing the alignment, anti-alignment, and repulsion effects. It is formulated
 667 in the following form (Grossmann et al., 2014, 2015):

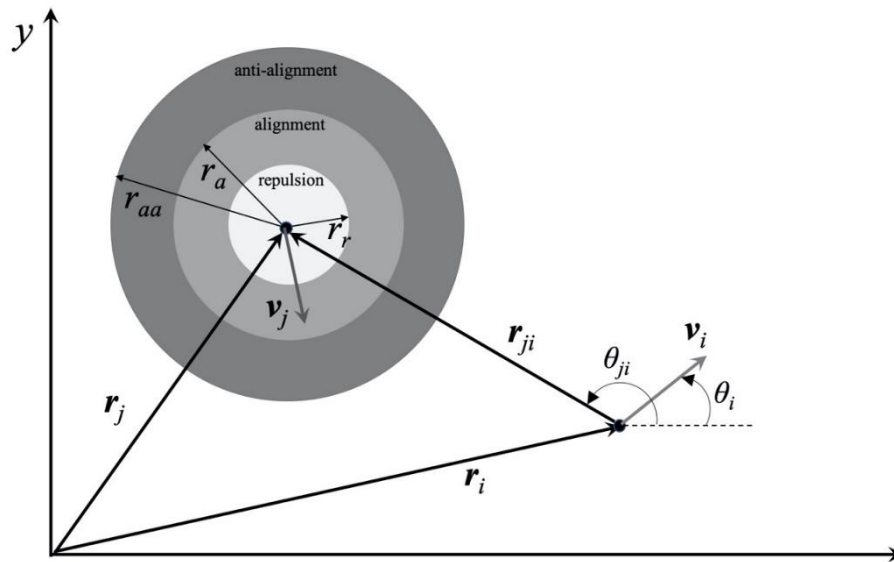
$$F_\theta(\mathbf{r}_{ji}, \hat{\mathbf{p}}_i, \hat{\mathbf{p}}_j) = k_r \mathcal{H}(r_r - r_{ji}) \sin(\theta_i - \theta_{ji}) + \mu \sin(\theta_j - \theta_i) \quad (3)$$

668 k_r is the magnitude of the constant repulsion interaction that applies over a distance of r_r around
 669 the particle (Figure S2). The second term in Eq. (3) represents the alignment and anti-alignment

670 effects, which operate over a range of r_a and r_{aa} , respectively. The magnitude of the aligning
 671 interaction μ is distance-dependent and is defined as (Grossmann et al., 2014, 2015):

$$\mu = \begin{cases} \mu^+ (1 - (r_{ji}/r_a)^2) & 0 \leq r_{ji} \leq r_a \\ -\mu^- \frac{4(r_{ji} - r_a)(r_{aa} - r_{ji})}{(r_{aa} - r_a)^2} & r_a \leq r_{ji} \leq r_{aa} \end{cases} \quad (4)$$

672 where μ^+ and μ^- are the strength of alignment and anti-alignment interactions, respectively.



673
 674 **Figure S2 | Schematic of the zonal pair-wise interaction model showing anti-alignment, alignment,**
 675 **and repulsion zones with the corresponding interaction radii r_{aa} , r_a , and r_r .**

676

677 We numerically integrate Eqs. (1) and (2) using the first-order Euler scheme. Initially, the particles
 678 are randomly distributed with random orientations. The integration time step Δt is selected
 679 sufficiently small to ensure both numerical stability and also independence of long-term statistics
 680 from Δt . The simulation time is set long enough to let the system reach a dynamic steady-state.
 681 The interaction of particles with the bounded circular domain is modeled via a reflective boundary
 682 condition.

683 Assessment of simulation parameters

684 Swarming cells secrete large amounts of surface-active compounds that modify the surface tension
685 locally(Fauvart et al., 2012; Ke, Hsueh, Cheng, Wu, & Liu, 2015), as well as micro-viscosity of
686 the fluid(Copeland & Weibel, 2009), which along with the formation of intercellular bundles
687 between neighboring cells, can enhance the cohesive interaction and alignment in swarmer cells.
688 Thus, simulation parameters must be chosen to capture different behaviors between the planktonic
689 and swarmer cells.

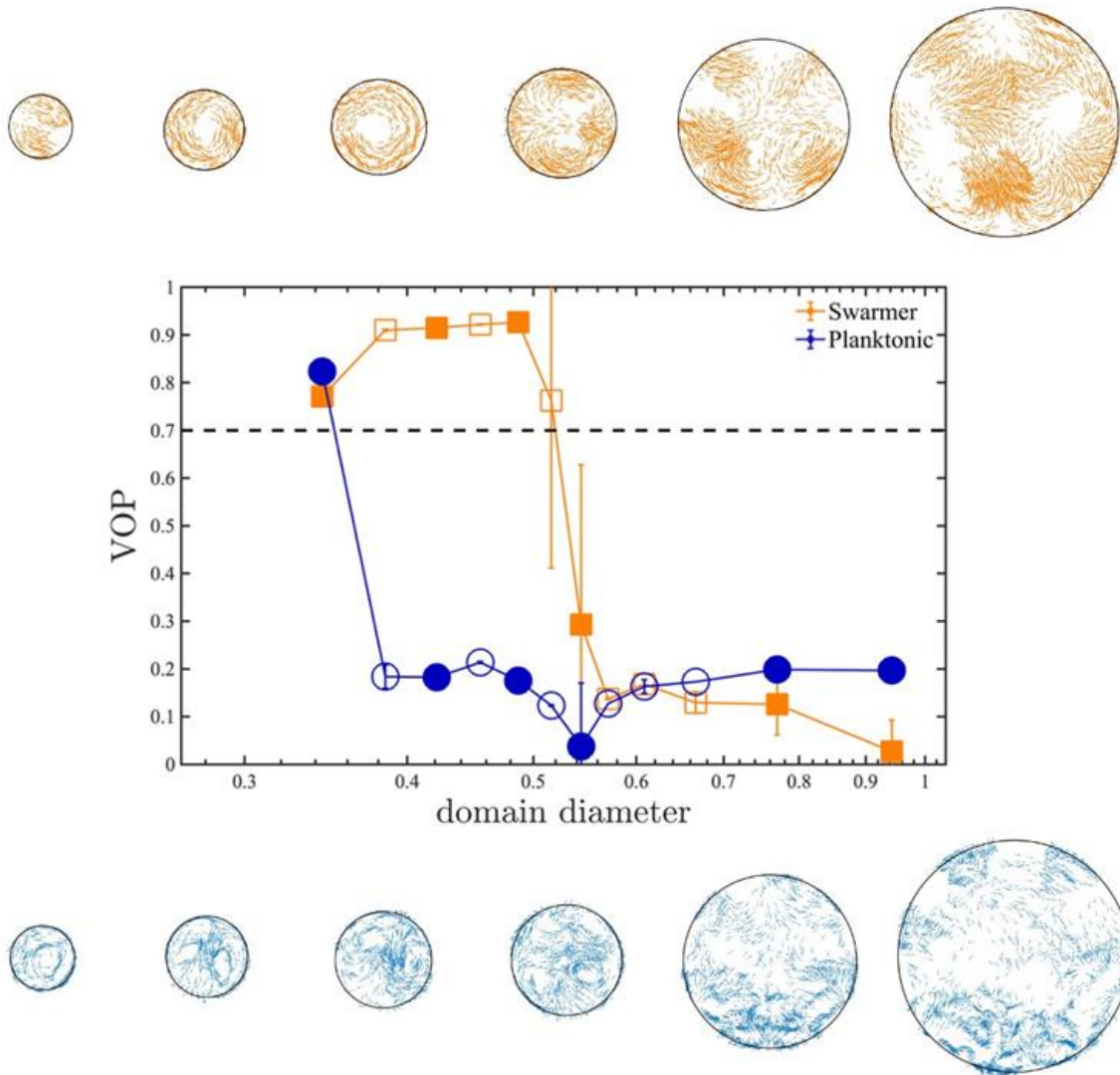
690 Two different sets of interaction parameters have been used to differentiate the swarming and
691 planktonic cases, and these parameters are summarized in Table S1. The values are unitless. We
692 set the exclusion parameters k_{ex} and d_{ex} to fixed values of 0.02 and 0.035, respectively. It is also
693 assumed that particles only experience a rotational diffusion D_r of 0.75. The simulations for both
694 swarming and planktonic forms have been studied at two particle densities $\rho = N/A_{dom}$, where N is
695 the number of particles, and A_{dom} is the simulation domain area. In the high-density case, $\rho = 4300$,
696 and in the dilute case, we set $\rho = 235$. In the dilute case, to further minimize the boundary effects,
697 we replace the bounded domain with a periodic boundary.

698

		Swarming	Planktonic
repulsion	k_r	2	3
	r_r	0.05	0.08
alignment	m^+	0.5	0.2
	r_a	0.2	0.2
Anti-alignment	m^-	0.5	4.0
	r_{aa}	0.25	0.25

Table S1 | Simulation parameters used for Swarming and Planktonic cases

699 The simulation results at high particle density $\rho = 4300$ for some representative confinement sizes
700 are shown in Figure S3. As Fig. S3 illustrates, the macroscopic behavior of both swarming and
701 planktonic cells is affected by the confinement size. The corresponding change in Vortex Order
702 Parameter (VOP) marks the transition from a single vortex to multiple swirls. Compared to the
703 swarming case, the higher values of anti-alignment and repulsive interactions in the planktonic
704 case trigger an earlier onset of the transition.



705

706 **Figure S3 | Representative patterns at different sizes of the bounded domain.** Top row: Swarming;
707 Bottom row: Planktonic. The corresponding domain sizes and VOP values are marked as filled symbols.
708 The particle density is kept constant as the area of the simulated region increases. Simulation parameters
709 are based on the values summarized in Table S1. $\rho = 4300$ in all cases.
710

711 The set of simulation parameters in Table S1 implies that (1) alignment interactions in planktonic
712 cells are suppressed via lower alignment and higher anti-alignment magnitudes, and (2) the
713 repulsive interaction in planktonic cells is more pronounced, in terms of higher values of the
714 magnitude and range of repulsive torque. Despite the empirical nature of these parameter values,
715 we found them to capture the competing interactions between planktonic and swarmer cells. The

716 simulation results provide valuable physical insights as the patterns predicted closely resemble the
717 experimental observation. More advanced real-time visualization of bundling dynamics in
718 swarmer cells(Copeland & Weibel, 2009), along with biochemical characterization of the bacterial
719 fluids, and the micro-rheology measurements within local, extracellular polymeric
720 network(Guadayol et al., 2020) will shed light on the underlying nature of complex physical and
721 chemical interactions. These properties rely on experimental effort beyond the scope of this report.
722 If determined, they will facilitate the development of more comprehensive particle-based models.

723 **Supporting Movies**

724 All videos play in real-time, except for Movie S7 & S8, which were taken in 20 fps but compressed
725 to play in 30 fps.

726 **Movie S1: Confined swarming SM3 showing a single swirl motion pattern.** Swarming SM3
727 was confined in 74 μm diameter PDMS wells.

728 **Movie S2: Confined concentrated planktonic SM3 showing a turbulent motion pattern.**
729 Swimming SM3 was confined in 74 μm diameter PDMS wells.

730 **Movie S3: Diluted swarming SM3 colony.** The swarming SM3 colony edge was diluted by
731 adding a 50 μL water droplet. Clusters of bacteria cells formed rafts.

732 **Movie S4: Diluted swimming SM3 suspension.** Concentrated planktonic SM3 was diluted by
733 adding a 50 μL water droplet. Bacterial cells were observed to swim independently without
734 clustering.

735 **Movie S5: Numerical simulations of circularly confined SM3.** Swarming SM3 (left) and
736 concentrated planktonic SM3 were simulated in the well size of 0.48. The video shows a
737 representative confined motion pattern. Arrows indicate the moving direction of the particles.

738

739 **Movie S6: Numerical simulations of SM3 cells in open space.** Diluted swarming SM3 (left) and
740 planktonic SM3 were simulated without confinement, but with a periodic boundary condition. In
741 both cases, cell density is $\rho = 235$, and the arrows indicate the moving directions of the particles.

742 **Movie S7: Fluorescent beads motion on DSS induced colitic mouse intestine tissue.** The
743 unidirectional rotation motion in 38 μm diameter wells indicates the presence of swarming SM3
744 on the tissue surface.

745 **Movie S8: Fluorescent beads motion on normal mouse intestine tissue.** The random motion in
746 38 μm diameter wells indicates predominantly planktonic SM3 on the normal mice tissue surface.

747

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