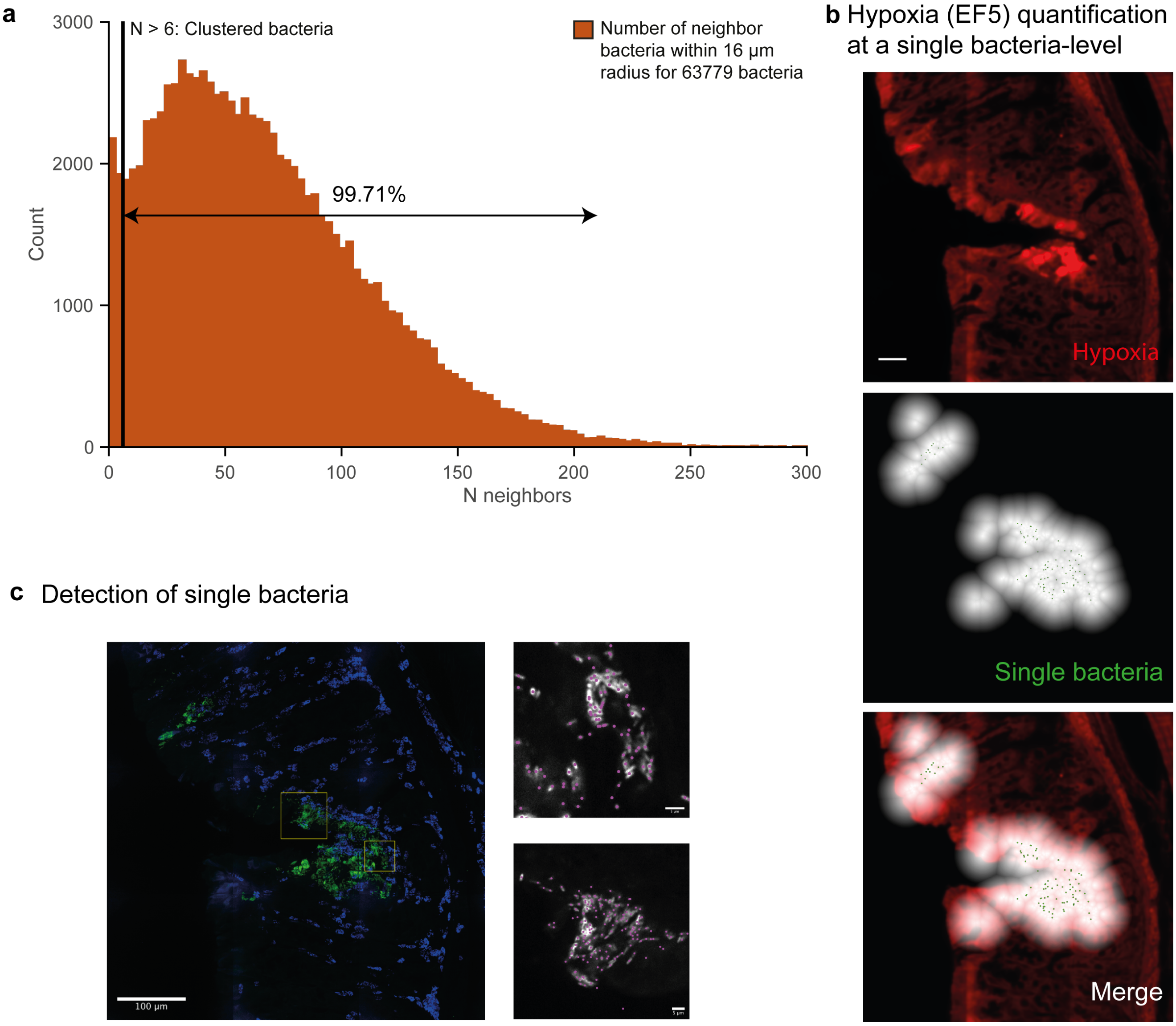
Supplementary information.

Content.

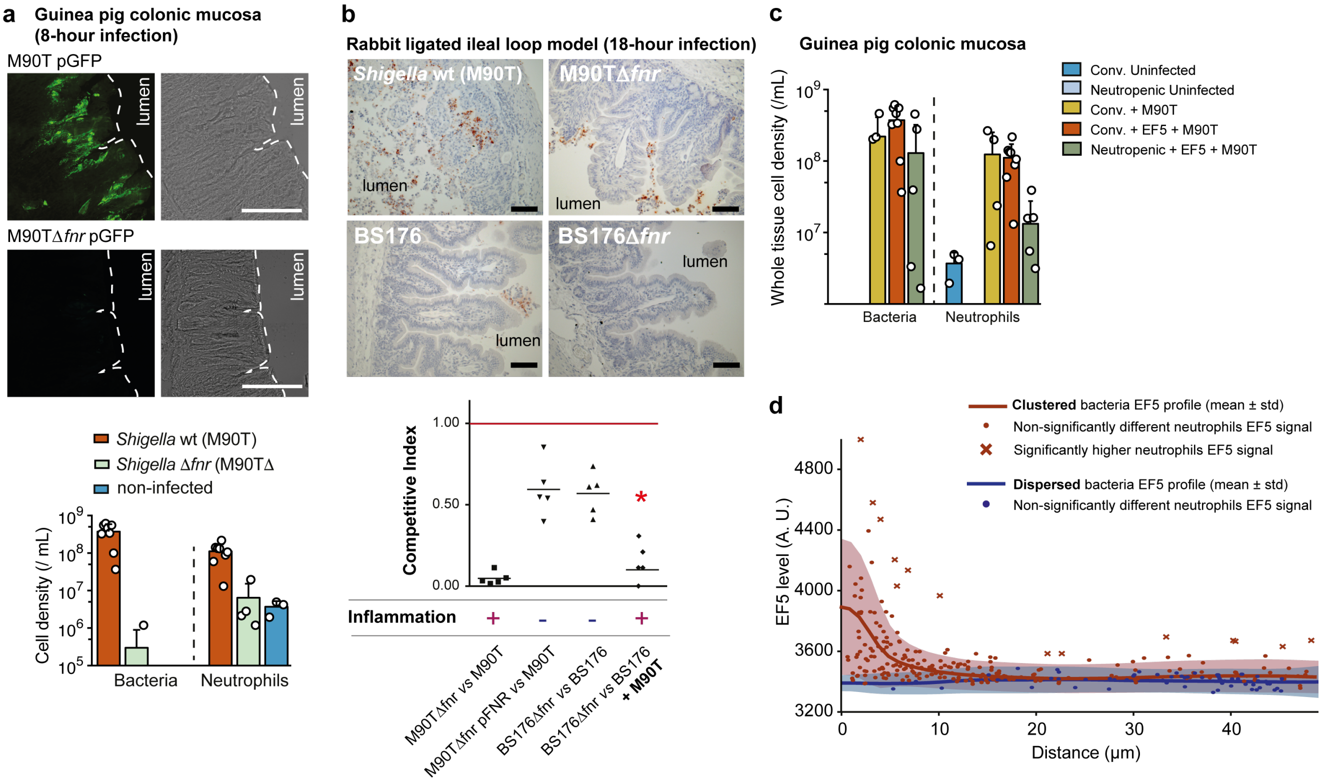
Supplementary figures.

Supplementary Figure 1. Single bacteria analysis.



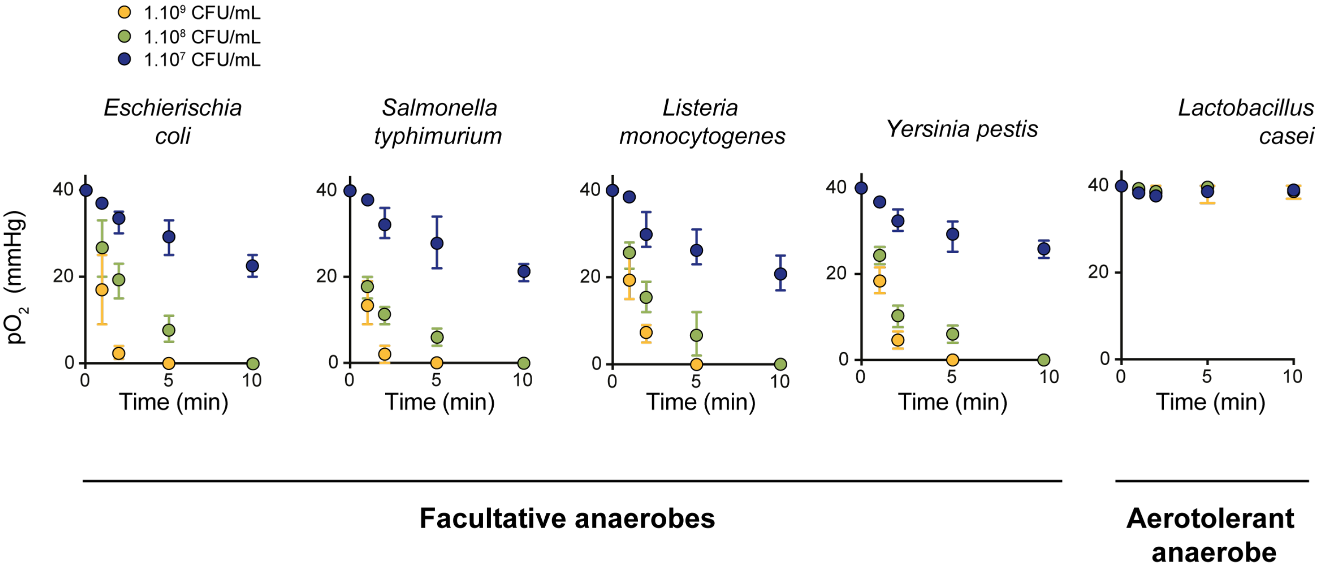
a. Histogram of the number of neighbors within 16 µm radius around each *Shigella* bacterium. The estimated size of an infection focus was determined by manual inspection to be roughly of 16 µm in radius. The histogram of the number of bacteria in this neighborhood is bimodal and displays two peaks, one close to N = 1 and one close to N = 30, with a local minimum at N = 6. We therefore classified bacteria as ‘clustered’ if they have more than 6 neighbors within a radius of 16 µm. **b.** EF5 signal in the central Z slice of the dataset used to generate Supplementary Figure 2b. Scale bar: 50 µm. For each pixel of a 3D image, the distance to the closest ‘’clustered’ bacteria is calculated thanks to the image distance transform. The distance here is displayed in grayscale from white (0-1 µm to the closest bacterium) to black (distance >= 50 µm). The EF5 pixel intensities are pooled together by their distance to the closest bacterium by bins of 1 µm width (see Methods). We then take the mean and standard deviation for each of these bins and report them as a function of the distance. See Supplementary Figure 2b. Green dots: single bacteria detected in this Z-slice and classified as ‘clustered’. Bacteria further away in Z by more than 1 µm or non-clustered are not shown. **c.** The images are acquired at a resolution sufficient to discriminate single bacteria, even when clustered. Left panel: same data as in panel b., maximum intensity projection showing the bacteria (green) and neutrophils (blue) channels. Scale bar: 100 µm. Right: Two insets (yellow box on the left panel) showing a single plane of the same data in the bacteria channels. Scale bars: 5 µm. Purple dots: results of automated bacteria detection, showing only bacteria centered in the current Z plane.

Supplementary Figure 2. FNR is essential for *S. flexneri* gut colonization and is hyper sensitive to the inflammatory environment *in vivo*.



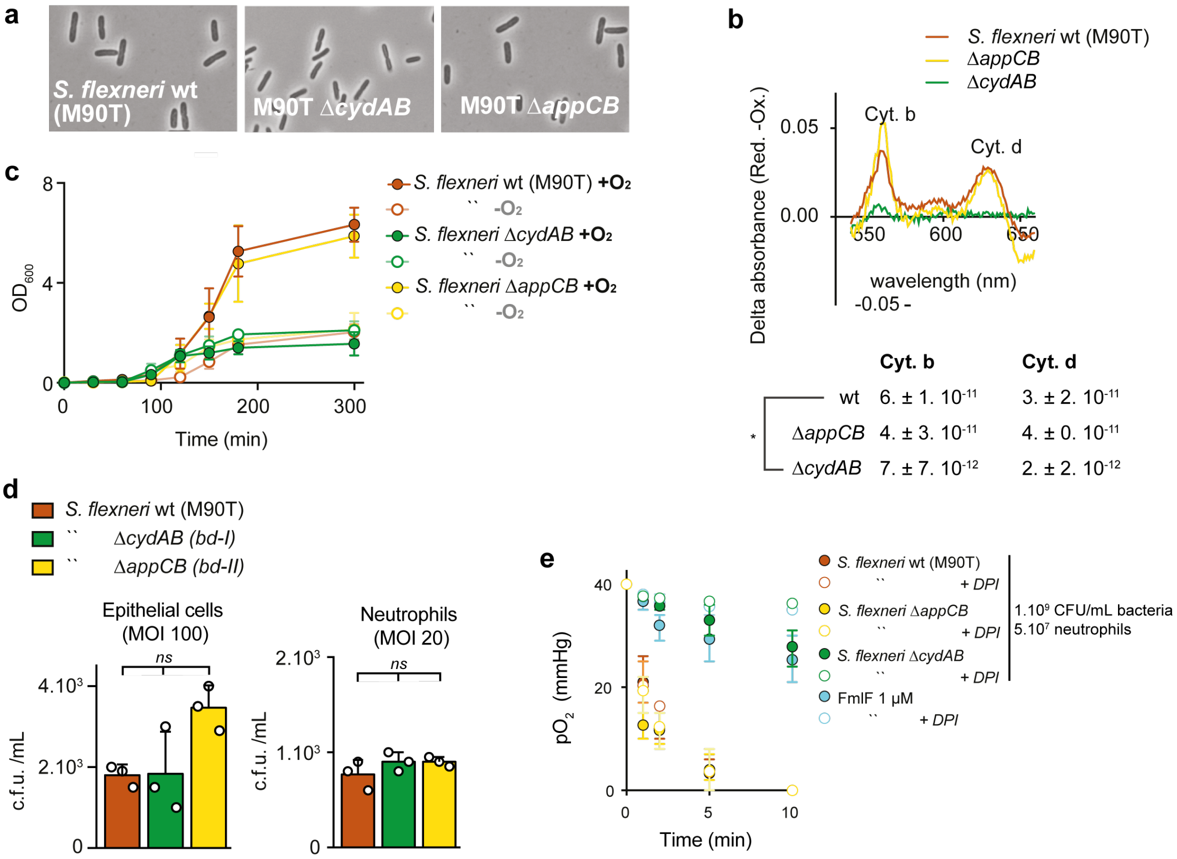
a. (**top**) Example image of colonic tissue during invasion by the *Shigella* M90T pGFP and the Δ*fnr* pGFP mutant strains (left: bacteria, right: transmitted light). No foci of infection were found in 4 animals infected by the latter strain. Scale bar: 50 µm. (**bottom**) Cell density (mean ± S.D.) calculated as the number of bacteria or neutrophils divided by the volume of the tissue locus imaged for each animal, expressed in mL. Y-axis uses logarithmic scale. Densities are found to be significantly different between M90T and Δfnr strains for bacteria ($3.7\ 10^8 \pm 2.1\ 10^8$ /mL, mean ± S.D, $n = 8$ loci imaged for M90T and $3.0\ 10^5 \pm 5.9\ 10^5$ /mL for Δfnr, $n = 4$ loci imaged, $p < 7\ 10^{-3}$, two-sided Student-t test) and neutrophils ($1.1\ 10^8 \pm 6.1\ 10^4$ /mL, $n = 8$ for M90T and $6.4\ 10^6 \pm 8.8\ 10^6$ /mL for Δfnr, $n = 4$ loci imaged, $p < 8\ 10^{-3}$). **b.** (**top**) Histological analysis of the rabbit GI tract infected with *S. flexneri* strains as indicated on each panel. There was marked destruction of the mucosal surface with loss of villi and infiltration of the submucosa with inflammatory cells following challenge with wild-type strain, M90T. These changes are less pronounced after challenge with the Δfnr mutant, BS176, and BS176Δfnr. Scale bars: 50 µm. (**bottom**) Competitive Indexes were calculated during rabbit gut co-infection (ileal loop luminal compartment, 5 loops per condition) with indicated *S. flexneri* strains. Horizontal bars: mean competitive index. M90T is a wild-type strain; BS176 is a plasma cured *S. flexneri* strain (non-invasive). FNR was inactivated in M90T and BS176 strains (M90TΔfnr and BS176Δfnr strains, respectively). The inflammatory statuses correspond to the observation of bloody tissue edema. When BS176 and BS176Δfnr were compared, inflammation was induced by co-infection with M90T (last column) (mean ± S.D. are calculated from n=5 biologically independent animal samples) (one-sided t-test, * indicates p=0.0003). **c.** Cell densities for neutropenic animals (mean ± S.D). Y-axis uses logarithmic scale. Data for the conventional animals infected by M90T in the presence of EF5 is taken from Supplementary Figure 2a-bottom. The bacteria density is roughly halved in neutropenic animals ($1.3\ 10^8 \pm 1.9\ 10^8$ /mL, $n = 6$ imaged loci, $p \sim 0.05$, two-sided Student-t test) compared to conventional animals. Infection by *Shigella* M90T triggers recruitment of neutrophils even in neutropenic animals ($1.3\ 10^7 \pm 1.4\ 10^7$ /mL, $n = 6$), though modestly compared to conventional animals ($1.1\ 10^8 \pm 6.1\ 10^7$ /mL, $n = 8$ imaged loci, $p < 3\ 10^{-3}$). **d.** Example plot of 16 replicates of local hypoxia profiles in the vicinity of individual bacteria. The EF5 level was measured in a single image as a function of the distance to the closest bacteria (see Methods and Supplementary Figure 1b), generating one plot for 'clustered' bacteria and one for 'dispersed' bacteria. For 'dispersed' bacteria, the hypoxia profile does not exhibit a peak at the bacteria location. However, for an individual bacterium in an infectious focus, the hypoxia profile peaks at the bacterium location and extends roughly 10 µm. Within the same image, the neutrophils were classified as closer to a 'clustered' bacterium or to a 'dispersed' bacterium, depending on the nearest bacterial neighbor. The EF5 intensity of these neutrophils is then reported on the proper hypoxia profile along with their distance to the closest bacterium. The local EF5 level of a neutrophil is then compared to the average EF5 profile generated by bacteria at that distance and classified as significantly higher (crosses, significance level at 5%) or not (dots) using a z-test.

Supplementary Figure 3. Oxygen consumption rate for facultative and tolerant anaerobes.



The rate of oxygen consumption was assessed as described in Figure 2e-g with *Eschierischia coli*, *Salmonella typhimurium*, *Listeria monocytogenes*, *Yersinia pestis* (facultative anaerobes), and *Lactobacillus casei* (aerotolerant anaerobe) wild-type strains. Oxygen tensions (expressed as mean ± S.D.) were assessed at indicated cell densities (*n* = 3 independent experiments).

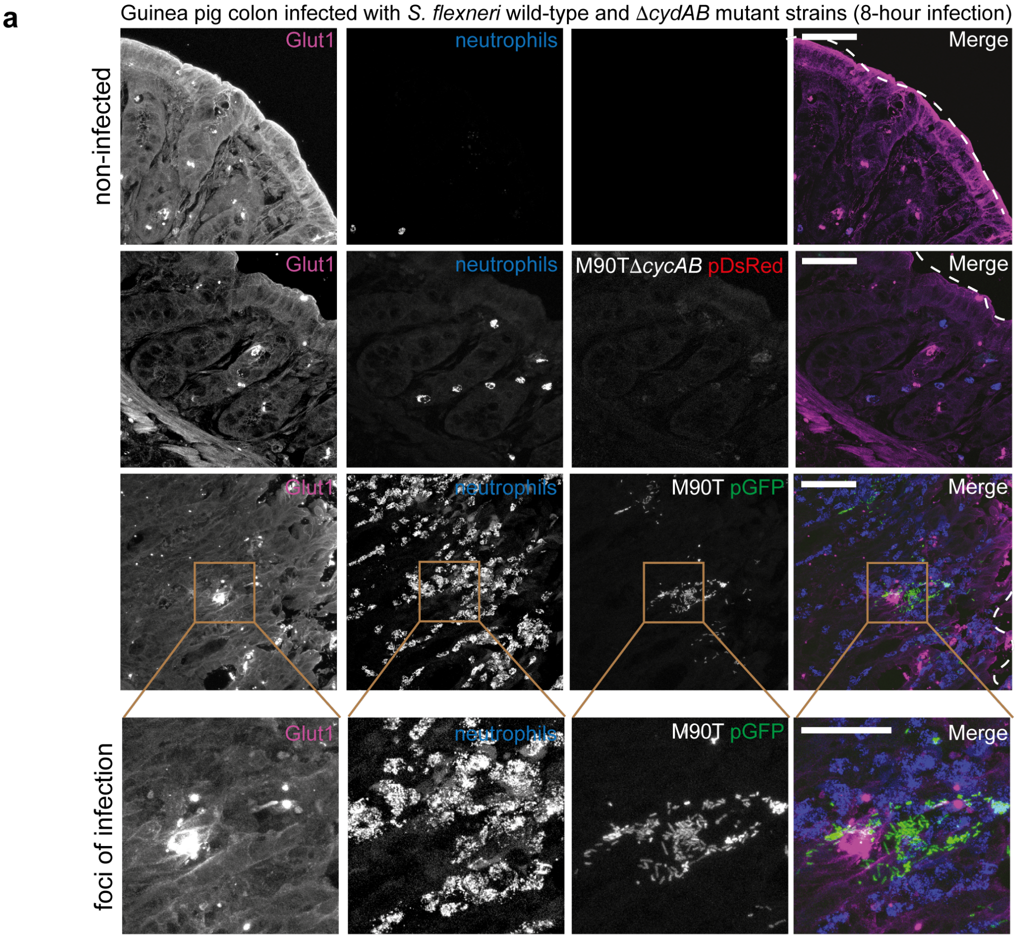
Supplementary Figure 4. Additional characterization of *S. flexneri* Δ*cydAB* and Δ*appBC* mutants.

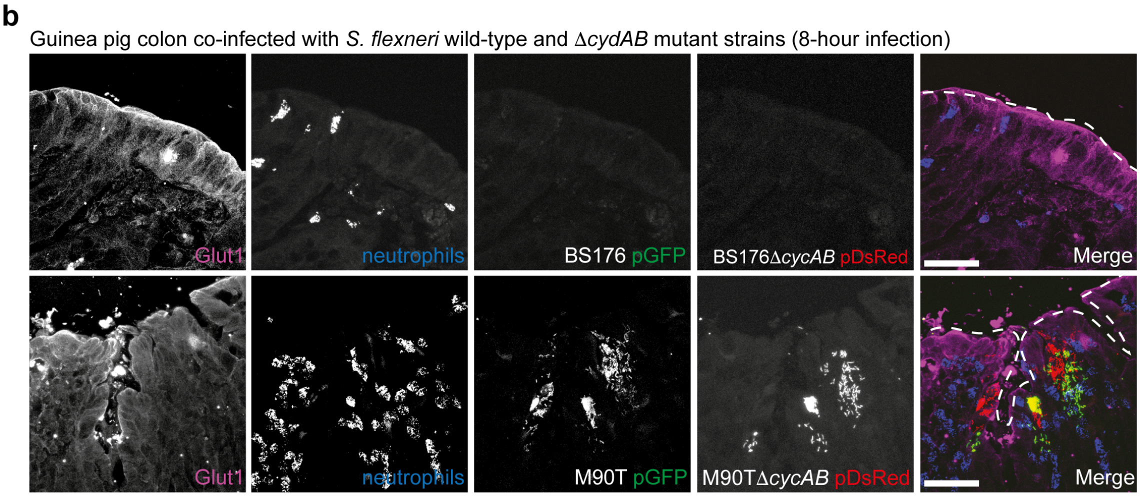


		Cyt. b	Cyt. d
*	wt	$6. \pm 1.\ 10^{-11}$	$3. \pm 2.\ 10^{-11}$
	Δ*appCB*	$4. \pm 3.\ 10^{-11}$	$4. \pm 0.\ 10^{-11}$
	Δ*cydAB*	$7. \pm 7.\ 10^{-12}$	$2. \pm 2.\ 10^{-12}$

a. Morphological analysis of *S. flexneri* 5a wild-type, Δ*cydAB* (*bd-I*), and Δ*appCB* (*bd-II*) mutant strains. Scale bar: 2 µm. This experiment was repeated independently three times with similar results. **b.** Cytochrome *b* and *d* expression defects in *S. flexneri* 5a wild-type, Δ*cydAB* (*bd-I*) mutant (representative experiment of 3 repetitions) and quantification (expressed as mol/OD_{600}, $n = 3$ independent experiments), compared to *S. flexneri* 5a wild-type and Δ*appCB* (*bd-II*) mutant strains (1-way ANOVA test, see Supplementary Table 9). **c.** *S. flexneri* 5a Δ*cydAB* (*bd-I*) have a growth defect in the presence of oxygen ($pO_2 = 160$ mmHg, mean ± S.D.) compared to *S. flexneri* 5a wild-type and Δ*appCB* (*bd-II*) mutant strains, not under anoxic conditions (as indicated by a 2-way ANOVA test, see Supplementary Table 10). **d.** *S. flexneri* 5a wild-type, Δ*cydAB* (*bd*-I), and Δ*appCB* (bd-*II*) mutant strains ability to invade epithelial cell (Hep2, MOI 100) and susceptibility to neutrophil phagocytosis (MOI 20) were not significantly different ($p = 0.42$ and $p = 0.3$ respectively, 1-way ANOVA test, see Supplementary Table 11). Colony forming units (c.f.u.) are reported with mean ± S.D. Cell infections occurred for 30 min under a $pO_2 = 40$ mmHg prior to intracellular bacteria quantification (gentamycin protection assays). **e.** Oxygen depletion in the presence of 8.10^7 neutrophils/mL showed that they do not significantly contribute to oxygen consumption when incubated with 1.10^9 CFU *S. flexneri* wild-type strain and Δ*appBC* (no significant differences between pO_2 curves with or without DPI for wild-type and Δ*appBC* strains, $p > 0.05$, mean ± S.D. over $n = 3$ independent experiments). In the presence of 1 µM FmlF and 1.10^9 CFU/mL *S. flexneri* Δ*cydAB* mutant, the contribution of neutrophils to oxygen consumption is statistically significant at late time points (10 min, 1-way ANOVA test, see Supplementary Table 12) and is inhibited by DPI (25 µM), although anoxia is not reached over the measurement period.

Supplementary Figure 5. GLUT-1 detection in guinea pig colonic mucosa infected by *S. flexneri* wild-type and mutant strains.


a Guinea pig colon infected with *S. flexneri* wild-type and Δ*cydAB* mutant strains (8-hour infection)


b Guinea pig colon co-infected with *S. flexneri* wild-type and Δ*cydAB* mutant strains (8-hour infection)

a. GLUT-1 was detected with a monoclonal antibody (magenta) in the colonic mucosa of naïve (non-infected) guinea pig or upon infection by *S. flexneri* wild-type strain (M90T pGFP, green), *S. flexneri* Δ*cydAB* pDsRed (red) mutant strain. Neutrophils were labeled with the MUB$_{40}$-Dylight405 marker (blue). Scale bar: 150 µm. This experiment was repeated independently three times with similar results. **b.** Similar experiments were conducted by co-infecting guinea pigs with plasmid cured BS176 pGFP/BS176Δ*cydAB* pDsRed or *S. flexneri* 5a wild-type (M90T pGFP)/M90TΔ*cydAB* pDsRed (red). As shown in Figure 3f, M90TΔ*cydAB* pDsRed was able to colonize the colonic mucosa upon co-infection with the wild-type strain. Plasmid-cured BS176 and BS176Δ*cydAB* remain in the luminal compartment. GLUT-1 was detected with a monoclonal antibody (magenta). Neutrophils were labeled with the MUB$_{40}$-Dylight405 marker (blue). Scale bar: 70 µm.

Supplementary tables.

Supplementary Table 1. Data generated in this work.

Condition	N fields imaged	N animals	N bacteria total	N clustered bacteria total	N dispersed bacteria total	N neutrophils total	Total tissue volume imaged (mm^3)
FNR + EF5	8	4	6	0	1	162	$22.2\ 10^{-3}$
M90T	8	4	11753	11400	28	5883	$41.8\ 10^{-3}$
M90T + EF5	37	9	63779	61119	186	19665	$163.1\ 10^{-1}$
M90T + EF5, Neutropenic	17	6	11372	6771	30	3289	$58.2\ 10^{-3}$
UI + EF5	6	3	0	0	0	66	$18.2\ 10^{-3}$
UI + EF5, Neutropenic	5	3	0	0	0	7	$15.1\ 10^{-3}$
Total	81	29	86910	79290	245	29072	$318.5\ 10^{-3}$

- FNR: *S. flexneri* Δfnr strain
- EF5: animals injected with EF5 prior challenge.
- M90T: *S. flexneri* 5a pGFP strain.
- Neutropenic: experiments done with neutropenic animals.
- UI: uninfected animals.

Supplementary Table 2. ANOVA test for hypoxia levels around dispersed or clustered bacteria.

Data displayed in Figure 1f.
EF5 levels.

Condition	Mean (A.U.)	Std (A.U.)	N bacteria
Clustered + EF5	3617	342	61119
Dispersed + EF5	3391	134	186
No EF5	3344	79	11400

ANOVA-1 table.

Source	Sum of squares	Degrees of freedom	Mean squares	F	Prob > F
Groups	$7.2\ 10^{8}$	2	$3.6\ 10^{8}$	3636	0
Error	$7.2\ 10^{9}$	72702	$9.9\ 10^{4}$		
Total	$7.9\ 10^{9}$	72704			

Post Hoc Tukey-test.

Condition 1	Condition 2	Lower conf. interval (95%, A.U.)	Diff. of means (A.U.)	Upper conf. interval (95%, A.U.)	*p*-value
No EF5	Clustered + EF5	-280.7	-273.2	-265.6	0
No EF5	Dispersed + EF5	-101.8	-47.2	7.4	0.1063
Clustered + EF5	Dispersed + EF5	171.7	226.0	280.2	0

Supplementary Table 3. Student-*t* test comparing hypoxia levels around bacteria in conventional *vs* neutropenic animals.

Data displayed in Figure 2b.
EF5 levels. Two-sided Student *t*-test comparing bacteria in conventional and neutropenic animals.

Condition		Mean (A.U.)	Std (A.U.)	N bacteria	*p*-values
Clustered bacteria	Conventional animals	3617	342	61119	< 0.0001
	Neutropenic animals	3506	126	6771	
Dispersed bacteria	Conventional animals	3391	134	186	> 0.37
	Neutropenic animals	3415	159	30	

Supplementary Table 4. Two-way ANOVA test for O_2 depletion rate as a function of bacteria density.

Data displayed in Figure 2g.

Left plot: O_2 depletion rate with a bacteria concentration of 5 10^9 / mL.

Two-way ANOVA table on *Condition* and *Time*. pO_2 in mmHg. *n*=3 independent experiments.

Source	Sum of squares	Degrees of freedom	Mean squares	F	Prob > F
Time	5.0 10^3	4	1.3 10^3	20.4	$< 10^{-8}$
Condition	8.8 10^3	2	4.4 10^3	74.5	$< 10^{-12}$
Error	2.3 10^3	38	61.3		
Total	1.6 10^4	44			

Condition: *S. flexneri*; *S. flexneri* + PMN; Naïve PMN.
Time: *0 min; 1 min; 2 min; 5 min; 10 min*.

Post Hoc Tukey-test along *Condition* dimension:

Condition 1	Condition 2	Lower conf. interval (95%, mmHg)	Diff. of means (mmHg)	Upper conf. interval (95%, mmHg)	*p*-value
S. flexneri	Naïve PMN	22.6	29.6	36.6	0
S. flexneri + PMN	Naïve PMN	22.6	29.6	36.6	0
S. flexneri + PMN	*S. flexneri*	-7.0	0	7.0	1

Middle plot: O_2 depletion rate with a bacteria concentration of 1 10^6 / mL.

Two-way ANOVA table on *Condition* and *Time*. pO_2 in mmHg. *n*=3 independent experiments.

Source	Sum of squares	Degrees of freedom	Mean squares	F	Prob > F
Time	421	4	105	24.7	0
Condition	43.2	1	43.2	10.1	< 0.01
Error	102	24	4.27		
Total	567	29			

Condition: *S. flexneri*; *S. flexneri* + PMN.
Time: *0 min; 1 min; 2 min; 5 min; 10 min.*

Post Hoc Tukey-test: For each comparison *S. flexneri vs S. flexneri* + PMN at equal time, we find $p \sim 0.09$.

Right plot: O_2 depletion rate with a heat-killed bacteria concentration of $5\ 10^9$ / mL.

Two-way ANOVA table on *Condition* and *Time*. pO_2 in mmHg. *n*=3 independent experiments.

Source	Sum of squares	Degrees of freedom	Mean squares	F	Prob > F
Time	414	4	35.3	11.3	0
Condition	19.2	1	19.2	6.13	0.02
Error	75.1	24	3.13		
Total	235.5	29			

Condition: *S. flexneri* heat-killed*; S. flexneri* heat-killed *+ PMN.*
Time: *0 min; 1 min; 2 min; 5 min; 10 min.*

Post Hoc Tukey-test: For each comparison *S. flexneri* heat-killed *vs S. flexneri* heat-killed *+ PMN* at equal *time*, we find $p \sim 0.33$.

Supplementary Table 5. Two-way ANOVA test for O_2 depletion rate for *S. flexneri* mutants.

Data displayed in Figure 3b.
Two-way ANOVA table, on *Condition* and *time*. pO_2 in mmHg. 3 independent experiments.

Source	Sum of squares	Degrees of freedom	Mean squares	F	Prob > F
Time	4199	4	1049	20.5	$< 10^{-8}$
Condition	4402	2	2201	43.1	$< 10^{-9}$
Error	1942	38	51.1		
Total	10589	44			

Condition: *S. flexneri* WT; Δ*appCB*; Δ*cydAB*.
Time: *0 min; 1 min; 2 min; 5 min; 10 min*.

Post Hoc Tukey-test along *Condition* dimension:

Condition 1	Condition 2	Lower conf. interval (95%, mmHg)	Diff. of means (mmHg)	Upper conf. interval (95%, mmHg)	*p*-value
ΔappCB	***ΔcydAB***	-25.8	-19.4	-13.0	0
Δ*appCB*	*S. flexneri* WT	-3.50	2.87	9.23	0.52
ΔcydAB	*S. flexneri* WT	15.9	22.3	28.6	0

Supplementary Table 6. Stools found in the distal colon after infection.

Data displayed in Figure 3d.
Varying infection agent. Conditions: *non-infected, infected by M90T, infected by mutant ΔcydAB, infected by mutant ΔappCB.*
Number of stools in the distal colon:

Condition	Mean	Std	N animals
M90T	0.0	0.0	5
Non-infected	8.0	0.7	5
ΔappCB	0.0	0.0	5
ΔcydAB	7.8	0.4	5

One-way ANOVA table.

Source	Sum of squares	Degrees of freedom	Mean squares	F	Prob > F
Groups	321	3	104	594.6	$< 10^{-15}$
Error	2.8	16	0.18		
Total	315	19			

Post Hoc Tukey-test.

Condition 1	Condition 2	Lower conf. interval (95%)	Diff. of means	Upper conf. interval (95%)	*p*-value
M90T	Non-infected	-8.8	-8	-7.2	$< 10^{-8}$
M90T	*ΔappCB*	-0.8	0	0.8	1
M90T	*ΔcydAB*	-8.6	-7.8	-7.0	$< 10^{-8}$
Non-infected	*ΔappCB*	7.2	8	8.6	$< 10^{-8}$
Non-infected	*ΔcydAB*	-0.6	0.2	1.0	0.87
ΔappCB	*ΔcydAB*	-8.6	-7.8	-7.0	$< 10^{-8}$

Supplementary Table 7. Infection foci per mm in the distal colon.

Data displayed in Figure 3e.
Varying infection agent. Conditions: *non-infected, infected by M90T, infected by mutant ΔcydAB, infected by mutant ΔappCB.*
Number of foci / mm in the distal colon:

Condition	Mean	Std	N animals
M90T	17.0	5.1	5
Non-infected	0.0	0.0	5
Δ*appCB*	22.6	5.3	5
Δ*cydAB*	2.2	2.0	5

One-way ANOVA table.

Source	Sum of squares	Degrees of freedom	Mean squares	F	Prob > F
Groups	1839	3	613	41.6	$< 10^{-7}$
Error	236	16	14.8		
Total	2075	19			

Post Hoc Tukey-test.

Condition 1	Condition 2	Lower conf. interval (95%)	Diff. of means	Upper conf. interval (95%)	*p*-value
M90T	Non-infected	10.0	17.0	23.9	$< 10^{-4}$
M90T	Δ*appCB*	-12.5	-5.6	1.35	0.14
M90T	Δ*cydAB*	7.9	14.8	21.7	$< 10^{-4}$
Non-infected	Δ*appCB*	-29.5	-22.6	-15.7	$< 10^{-6}$
Non-infected	Δ*cydAB*	-9.15	-2.20	4.75	0.80
Δ*appCB*	Δ*cydAB*	13.5	20.4	27.34	$< 10^{-5}$

Supplementary Table 8. O_2-dependent modulation of *S. flexneri* Type III secretion system (T3SS).

Data displayed in Figure 4b.
Conditions: with or without O_2 (+/-O_2), with or without Congo-Red (+/-CR).
M90T pTSAR - GFP intensity (A.U.)

Condition	Mean (A.U.)	Std (A.U.)	N
-O_2 -CR	561.8	81.7	103
-O_2 +CR	579.1	91.0	110
+O_2 -CR	579.8	120.5	104
+O_2 +CR	1305.7	264.5	103

One-way ANOVA table.

Source	Sum of squares	Degrees of freedom	Mean squares	F	Prob > F
Groups	$4.1\ 10^7$	3	$1.4\ 10^7$	567.2	0
Error	$1.0\ 10^7$	416	$2.4\ 10^4$		
Total	$5.1\ 10^7$	419			

Post Hoc Tukey-test.

Condition 1	Condition 2	Lower conf. interval (95%, A.U.)	Diff. of means (A.U.)	Upper conf. interval (95%, A.U.)	*p*-value
-O_2 -CR	-O_2 +CR	-65.2	-10.5	44.2	> 0.9
-O_2 -CR	+O_2 -CR	-77.0	-21.6	33.9	> 0.7
-O_2 +CR	+O_2 -CR	-65.6	-11.1	43.5	> 0.9
-O_2 -CR	**+O_2 +CR**	-792.7	-737.1	-681.5	0
-O_2 +CR	**+O_2 +CR**	-781.3	-726.6	-671.9	0
+O_2 -CR	**+O_2 +CR**	-771.0	-715.5	-660.1	0

Supplementary Table 9. Cytochrome *b* and *d* expression defects in *S. flexneri* mutants.

Data displayed in Supplementary Figure 4b. mol/OD600.

Cytochrome b.

One-way ANOVA table. 3 independent experiments.

Source	Sum of squares	Degrees of freedom	Mean squares	F	Prob > F
Groups	3.4 10-21	2	$1.7\ 10^{-21}$	6.2	0.035
Error	$1.6\ 10^{-21}$	6	$2.7\ 10^{-22}$		
Total	$5.0\ 10^{-21}$	8			

Post Hoc Tukey-test.

Condition 1	Condition 2	Lower conf. interval (95%, A.U.)	Diff. of means (A.U.)	Upper conf. interval (95%, A.U.)	*p*-value
ΔappCB	*ΔcydAB*	$-0.6\ 10^{-11}$	$3.5\ 10^{-11}$	$7.7\ 10^{-11}$	0.088
ΔappCB	WT	-5.1 10-^{11}	$-1.0\ 10^{-11}$	$3.2\ 10^{-11}$	0.762
ΔcydAB	WT	$-8.6\ 10^{-11}$	$-4.5\ 10^{-11}$	$-0.4\ 10^{-11}$	0.036

Cytochrome d.

One-way ANOVA table. 3 independent experiments.

Source	Sum of squares	Degrees of freedom	Mean squares	F	Prob > F
Groups	$2.6\ 10^{-21}$	2	$1.3\ 10^{-21}$	7.2	0.026
Error	$1.1\ 10^{-21}$	6	$1.8\ 10^{-22}$		
Total	$3.7\ 10^{-21}$	8			

Post Hoc Tukey-test.

Condition 1	Condition 2	Lower conf. interval (95%, A.U.)	Diff. of means (A.U.)	Upper conf. interval (95%, A.U.)	*p*-value
ΔappCB	*ΔcydAB*	$0.7\ 10^{-11}$	$4.1\ 10^{-11}$	$7.5\ 10^{-11}$	0.023
ΔappCB	WT	$-1.9\ 10^{-11}$	$1.5\ 10^{-11}$	$4.8\ 10^{-11}$	0.430
ΔcydAB	WT	$-6.0\ 10^{-11}$	$-2.6\ 10^{-11}$	$0.8\ 10^{-11}$	0.112

Supplementary Table 10. ANOVA tests for the growth curves of mutant *vs* wild-type *S.flexneri* in the presence or absence of oxygen.

Data displayed in Supplementary Figure 4c.
Optical density, 3 independent experiments.
One-way ANOVA table.

	Source	Sum of squares	Degrees of freedom	Mean squares	F	Prob > F
T = 180 min	**Groups**	46.2	5	9.2	15.3	$7.7\ 10^{-5}$
	Error	7.3	12	0.6		
	Total	53.5	17			
T = 300 min	**Groups**	70.0	5	13.9	39.7	$4.7\ 10^{-7}$
	Error	4.2	12	0.4		
	Total	73.8	17			

Post Hoc Tukey-test:

Here we provide the comparisons by groups of conditions:

- group A: *S.flexneri* WT+O_2; *S.flexneri* Δ*appCB*+O_2.
- group B: *S.flexneri* WT-O_2; *S.flexneri* Δ*appCB*-O_2; *S.flexneri* ΔcydAB-O_2; ***S.flexneri* Δ*cydAB*+O_2**.

Pairwise comparisons with Tukey test for any member of one group against any member of the other group:

	180 min	*300 min*
Comparison	***p*-value**	***p*-value**
Within group A	> 0.9	> 0.9
Within group B	> 0.9	> 0.8
Group A *vs* group B	< 0.01	< 0.001

Supplementary Table 11. *S.flexneri* mutant ability to invade epithelial cells and susceptibility to phagocytosis by neutrophils.

Data displayed in Supplementary Figure 4d.
Conditions: *M90T (S.flexneri W.T.), mutant ΔcydAB, mutant ΔappCB.*

Left plot: Ability to invade epithelial cells.

c.f.u. / mL, Hep2, MOI 100.

Condition	Mean (c.f.u. /mL)	Std (c.f.u. / mL)	N
M90T	1800	55	3
ΔcydAB	3467	1041	3
ΔappCB	1833	245	3

One-way ANOVA table.

Source	Sum of squares	Degrees of freedom	Mean squares	F	Prob > F
Groups	$5.4\ 10^{6}$	2	$2.7\ 10^{6}$	5.61	0.042
Error	$2.9\ 10^{6}$	6	$4.9\ 10^{5}$		
Total	$8.4\ 10^{6}$	8			

Post Hoc Tukey-test.

Condition 1	Condition 2	Lower conf. interval (95%)	Diff. of means	Upper conf. interval (95%)	*p*-value
ΔappCB	*ΔcydAB*	-3379	-1633	112	0.064
ΔappCB	M90T	-1712	33	1779	1
ΔcydAB	M90T	-79	1667	3412	0.059

Right plot: Susceptibility to neutrophil phagocytosis.

c.f.u. / mL, MOI 20.

Condition	Mean (c.f.u. /mL)	Std (c.f.u. / mL)	N
M90T	767	153	3
ΔcydAB	900	100	3
ΔappCB	900	50	3

One-way ANOVA table.

Source	Sum of squares	Degrees of freedom	Mean squares	F	Prob > F
Groups	$3.6\ 10^5$	2	$1.7\ 10^5$	1.49	0.30
Error	$7.2\ 10^5$	6	$1.2\ 10^5$		
Total	$1.1\ 10^6$	8			

Post Hoc Tukey-test.

Condition 1	Condition 2	Lower conf. interval (95%)	Diff. of means	Upper conf. interval (95%)	*p*-value
ΔappCB	*ΔcydAB*	-274	0	273	1
ΔappCB	M90T	-140	133	407	0.36
ΔcydAB	M90T	-140	133	407	0.36

Supplementary Table 12. Oxygen depletion in the presence of neutrophils.

Data displayed in Supplementary Figure 4e.
Two-way ANOVA table, on *Condition* and *time*. pO_2 in mmHg. 3 independent experiments.

Source	Sum of squares	Degrees of freedom	Mean squares	F	Prob > F
Time	8627.0	4	2156.7	51.0	$4.7\ 10^{-24}$
Condition	13077	7	1868.2	44.2	$6.7\ 10^{-29}$
Error	4565.4	108	42.3		
Total	2629.6	119			

Condition: We split them in two groups to simplify pairwise comparison results display.

Group A	*Group B*
FmlF (1 mM)	*S. flexneri WT*
FmlF (1 mM) + DPI	*S. flexneri WT + DPI*
S. flexneri ΔcydAB	*S. flexneri ΔappCB*
S. flexneri ΔcydAB + DPI	*S. flexneri ΔaddCB + DPI*

Time: 0 min; 1 min; 2 min; 5 min; 10 min.

Post Hoc Tukey-test along Condition dimension:

Condition 1	**Condition 2**	***p*-value**
Any in Group A	Any in Group B	$< 10^{-7}$
Any in Group A	Any in Group A	> 0.3
Any in Group B	Any in Group B	> 0.9