# *Supplementary Material*

**Table S1.** Bacterial strains.

| Bacterial strain or plasmid | Relevant characteristics | Reference or source[a] |
|---|---|---|
| ***Clostridium botulinum*** | | |
| ATCC3502 | Group I, produces type A botulinum neurotoxin (BoNT/A) | (54) |
| NCTC2916 | Group I, produces BoNT/A, carries a silent gene for BoNT/B | (55) |
| 62A | Group I, produces BoNT/A | (55) |
| ATCC19397 | Group I, produces BoNT/A | (56) |
| 133-4803 | Group I, produces BoNT/B | (57) |
| 213B | Group I, produces BoNT/B | (57) |
| F Langeland | Group I, produces BoNT/F | (55) |
| Eklund 2B | Group II, produces BoNT/B | (55) |
| CB11/1-1 | Group II, produces BoNT/E | (58) |
| K126 | Group II, produces BoNT/E | (58) |
| Eklund 202F | Group II, produces BoNT/F | (55) |
| BKT2873 | Group III, produces BoNT/CD | (59) |
| **Other species** | | |
| *Clostridium sporogenes* NINF45 | Nontoxigenic, closely related to *C. botulinum* Group I | (31) |
| *Clostridium baratii* CCUG24033 | Nontoxigenic, Also called *C. botulinum* Group V, type strain | (31) |
| *Clostridium butyricum* BL86/13 | Also called C. botulinum Group VI, produces BoNT/E | DFHEH/QI |
| *Clostridium perfringens* ATCC13124 | Gas gangrene isolate, produces α-toxin | (60) |
| *Bacillus cereus* ATCC14579 | *B. cereus* type strain | ATCC |
| *Bacillus subtilis* 1012M15 | Derivative strain from *B. subtilis* 168 | (12) |
| *Listeria monocytogenes* EGD-e | Serotype 1/2a, causes listeriosis | (61) |
| *Staphylococcus aureus* ATCC12600 | *S. aureus* type strain | (62) |
| *Escherichia coli* 5 alpha | Derivative strain of *E. coli* DH5α | NEB |
| *Escherichia coli* Rosetta 2(DE3) pLysS | $F^-$ *ompT hsdS*$_B$($r_B^-$ $m_B^-$) *gal dcm* (DE3) pLysSpRARE2[3] ($Cam^R$) | Merck |

[a] DFHEH, Department of Food Hygiene and Environmental Health, Faculty of Veterinary Medicine, University of Helsinki, Finland. QI, Quadram Institute, United Kingdom. ATCC, American Type Culture Collection.

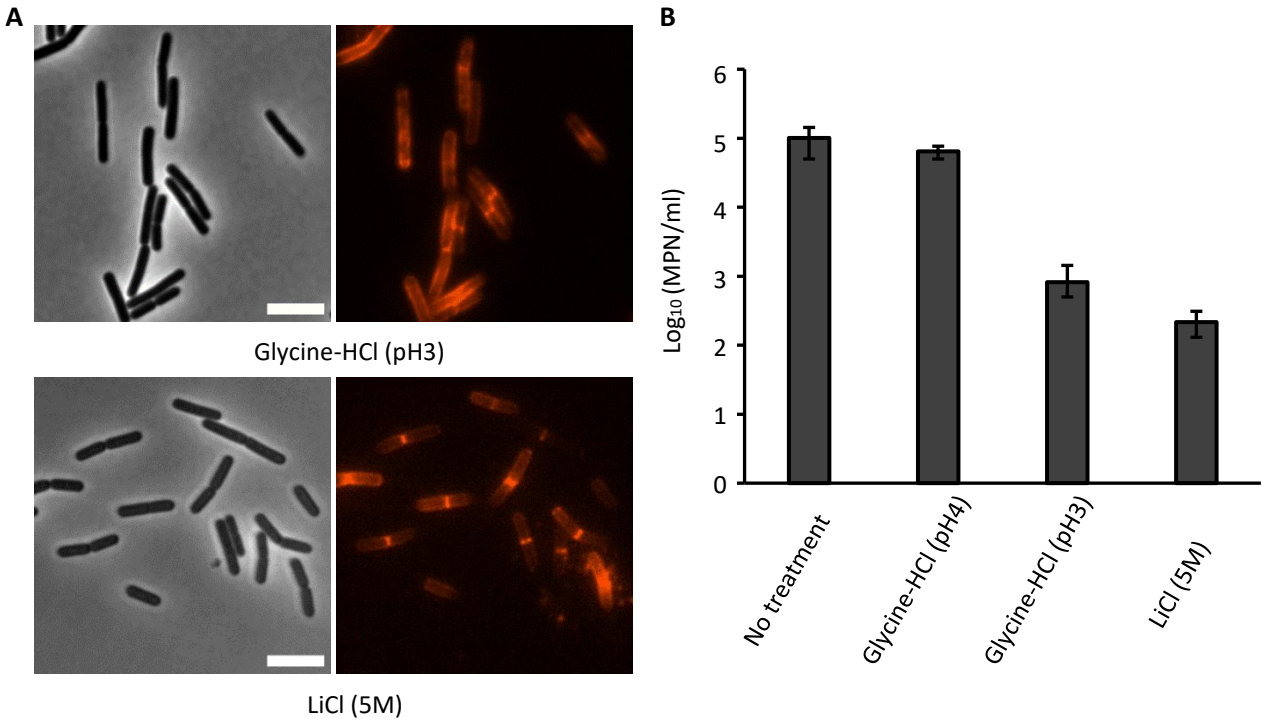
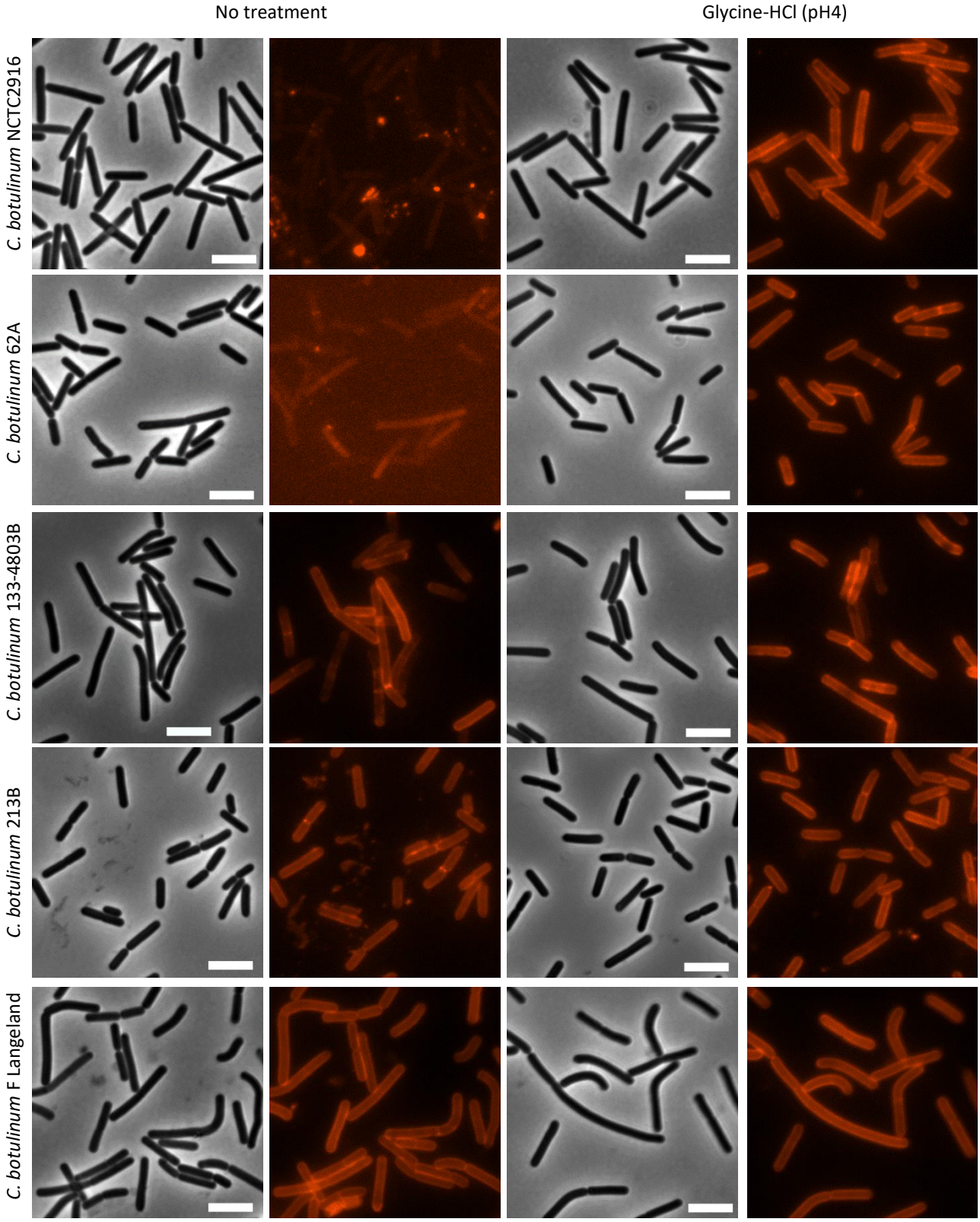



**Figure S1.** Treatment of *C. botulinum* ATCC3502 vegetative cells with glycine-HCl (pH3) and LiCl. (**A**) Representative fluorescence images showing the binding of mCherry-CBD to *C. botulinum* ATCC3502 cells after treated with 0.2M glycine-HCl (pH3) and 5M LiCl. Bars, 5 µm. (**B**) Most probable number (MPN) enumeration of equal fractions of *C. botulinum* ATCC3502 vegetative cells that were either no treatment or treated with 0.2 M glycine-HCl (pH4), glycine-HCl (pH3) or 5M LiCl for 1 min. Results are presented as means of three replicates ± standard deviations.


No treatment
Glycine-HCl (pH4)
C. botulinum NCTC2916
C. botulinum 62A
C. botulinum 133-4803B
C. botulinum 213B
C. botulinum F Langeland

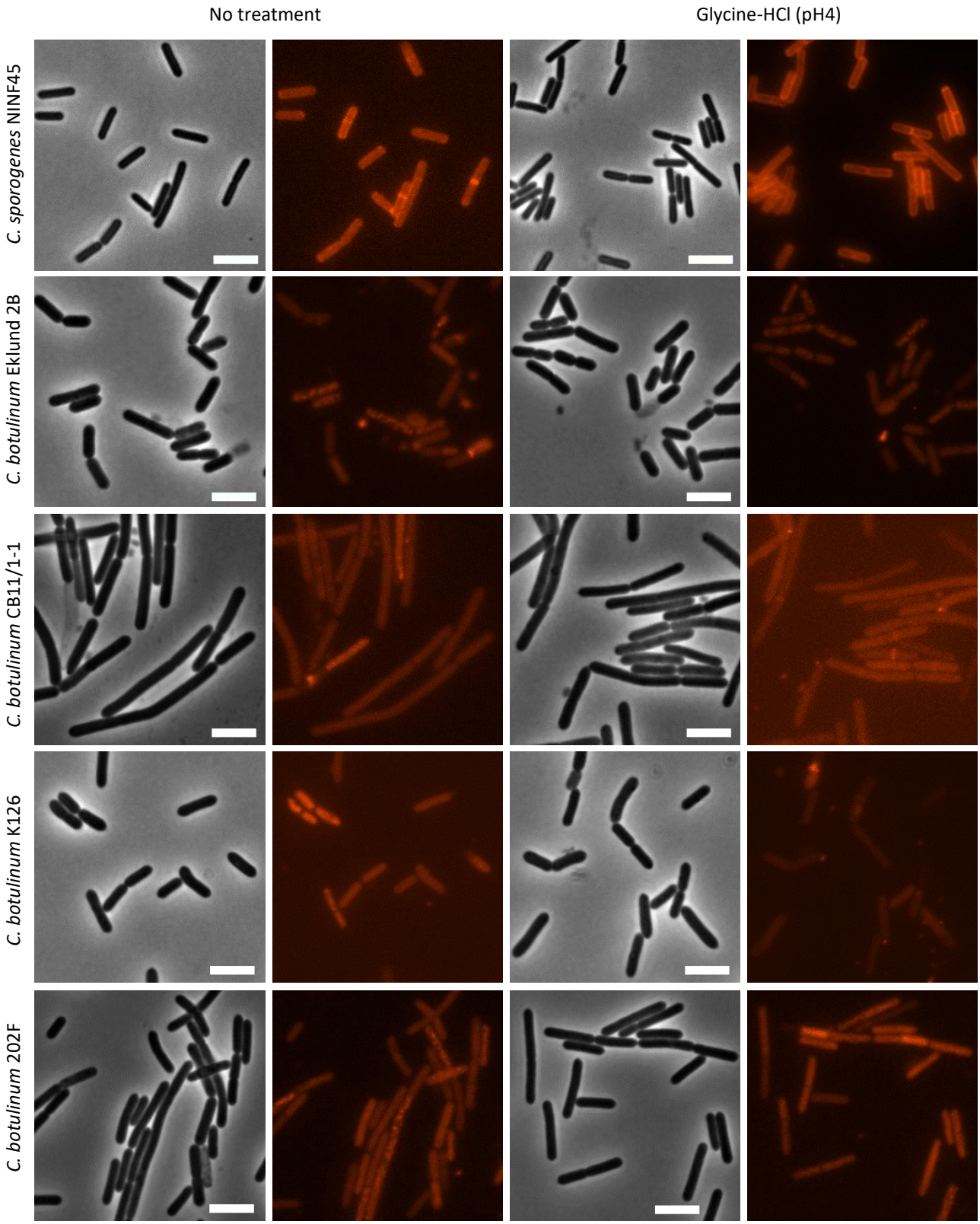



Figure S2 (continued)

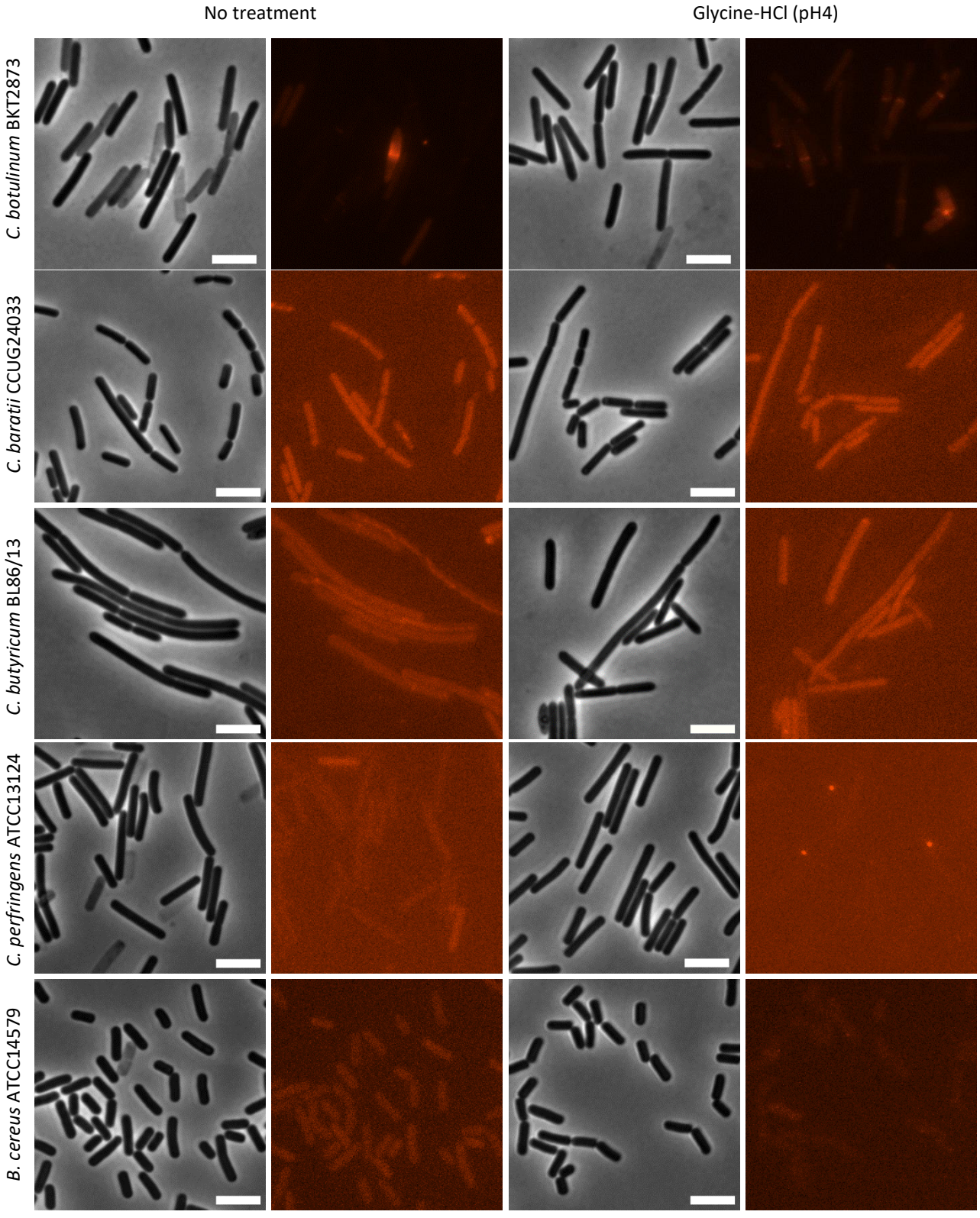



Figure S2 (continued)

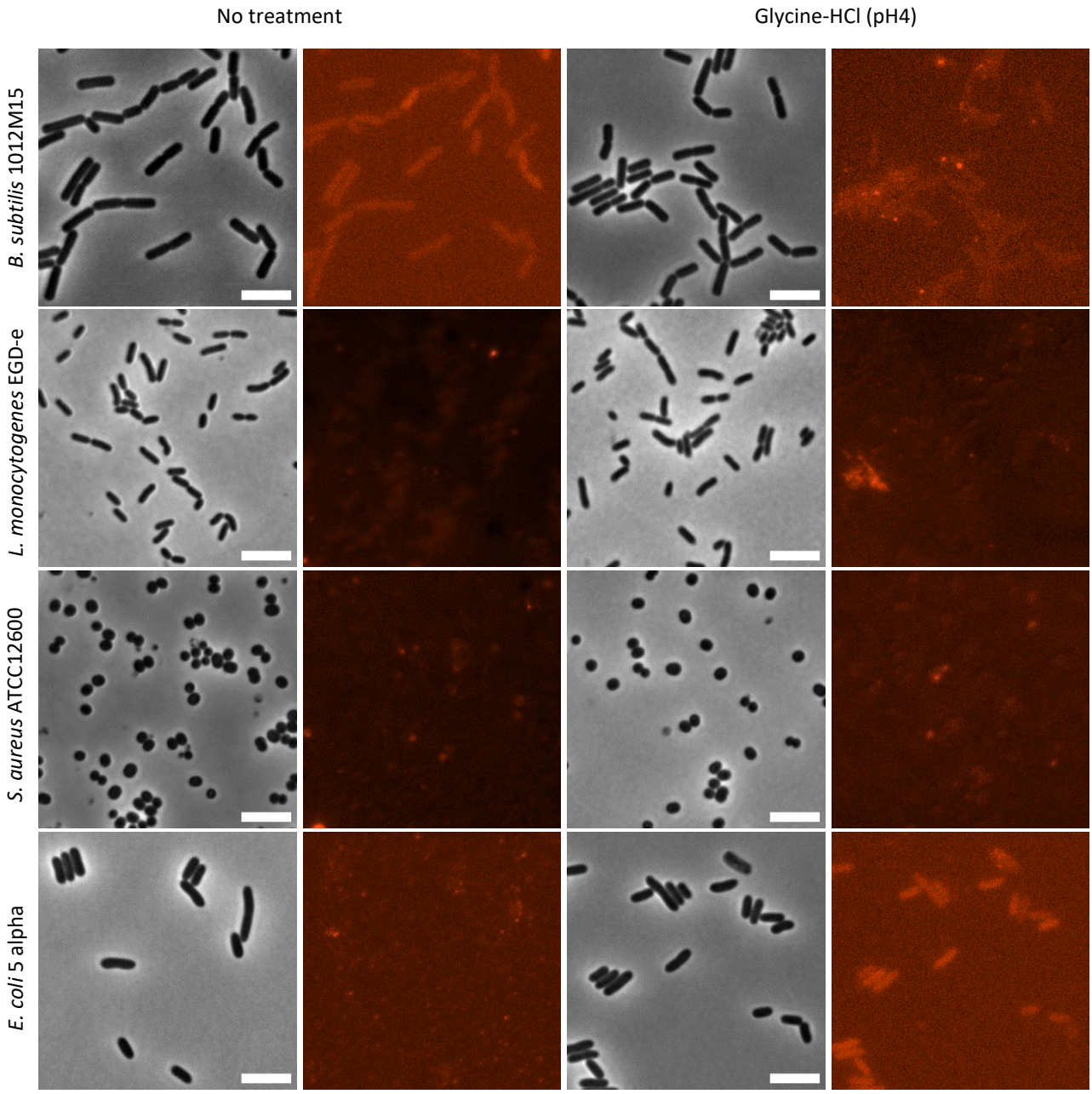



**Figure S2.** Representative fluorescence images of bacterial vegetative cells after incubation with mCherry-CBD. Before that, cells were either no treatment or treated with 0.2 M Glycine-HCl (pH 4) for 1 min. Bars, 5 μm.

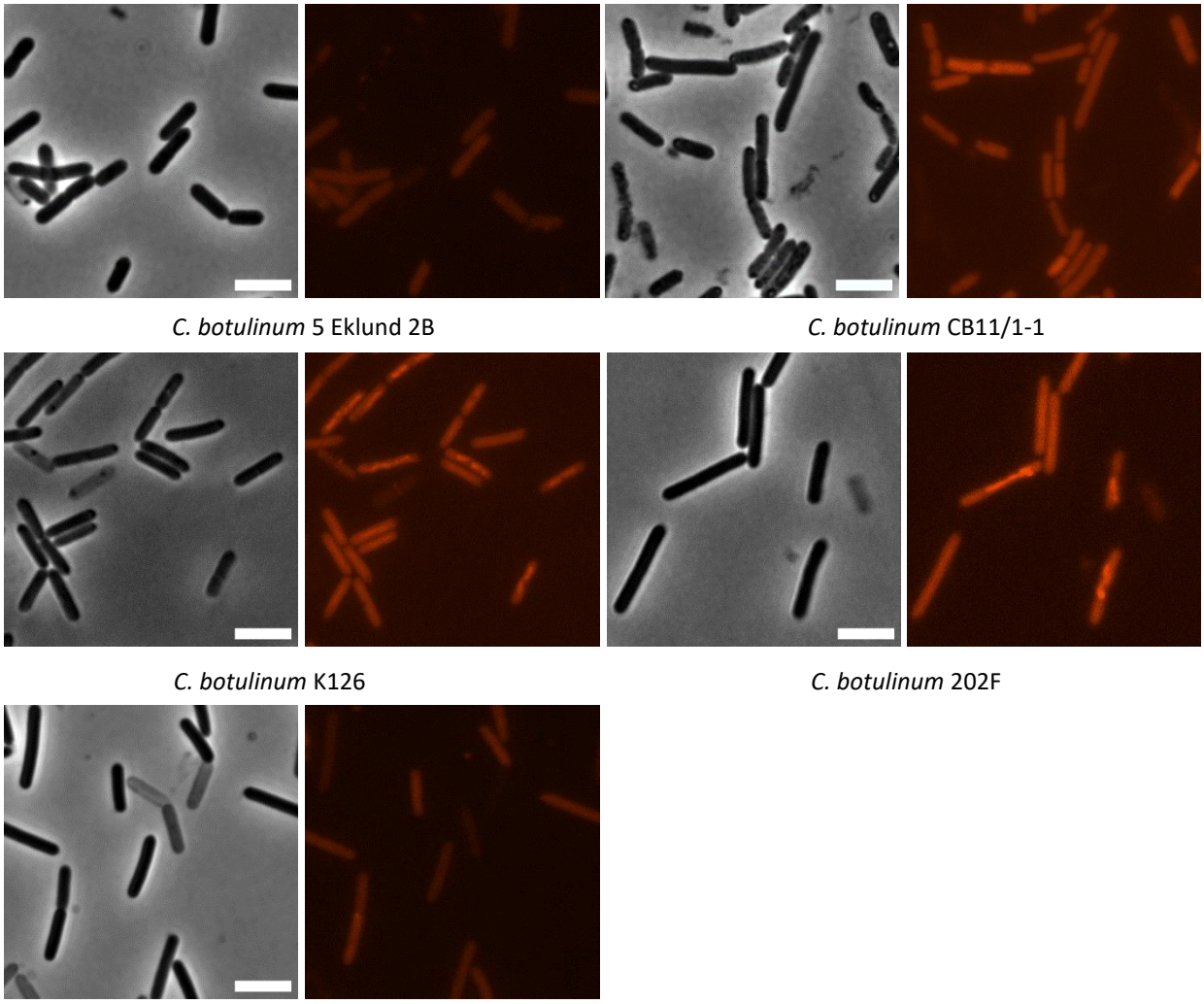



**Figure S3.** Representative images of *C. botulinum* Group II and Group III vegetative cells showing autofluorescence when viewed with a Texas Red filter.

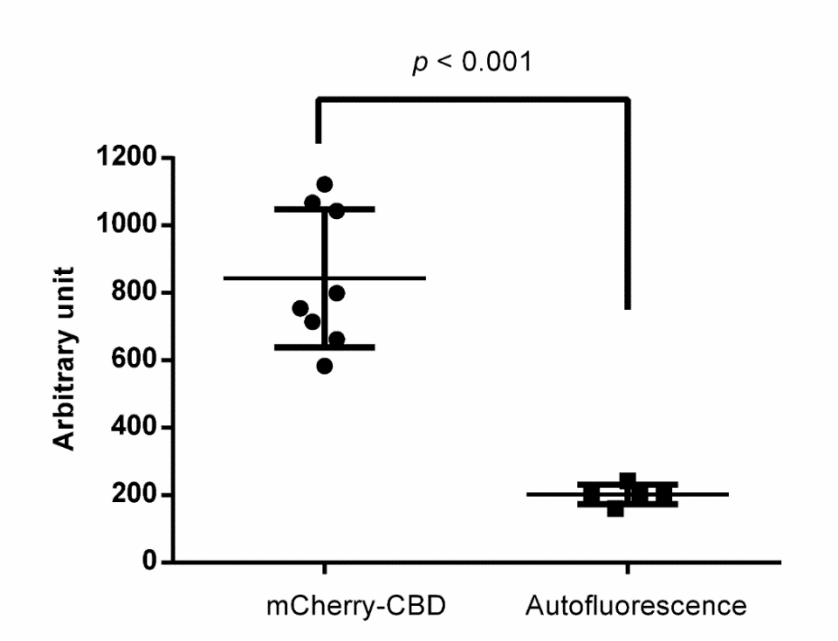



**Figure S4.** Comparison of fluorescence intensity of mCherry-CBD-labeled cells and autofluorescent cells. Fluorescence intensity of mCherry-CBD was measured from seven *C. botulinum* Group I strains and *C. sporogenes* NINF45. Autofluorescence intensity was measured from five *C. botulinum* Group II and Group III strains. Student's *t*-test was used for statistical analysis.

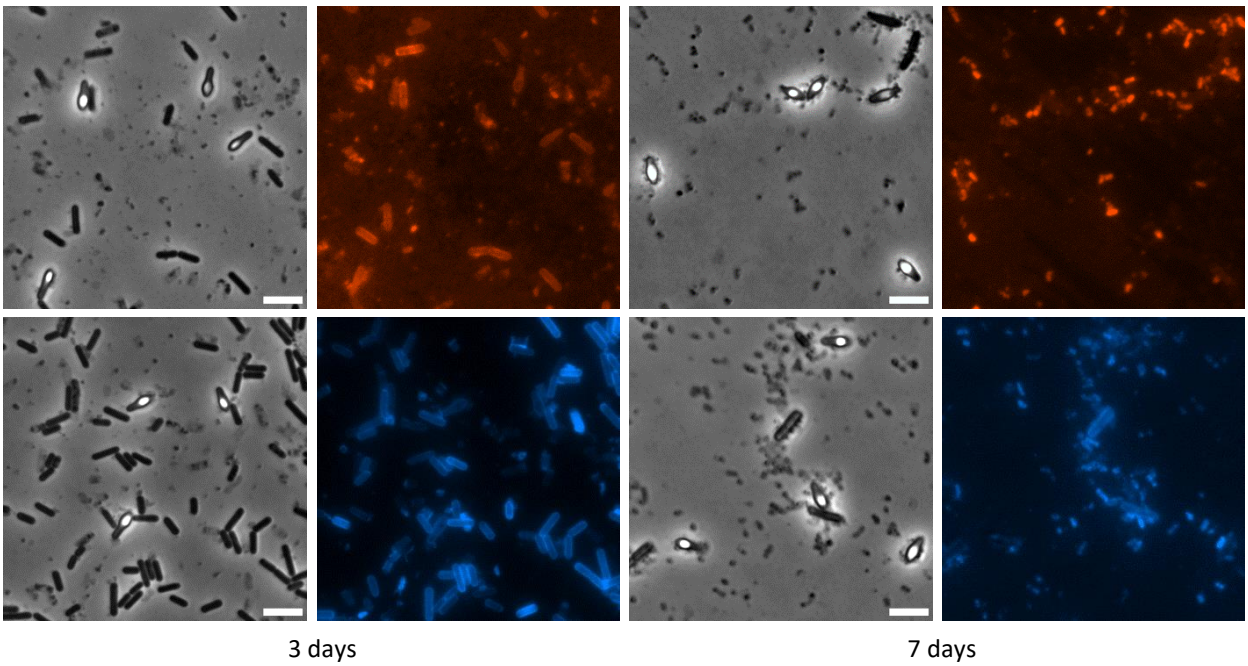



**Figure S5.** Representative fluorescence images of *C. botulinum* ATCC19397 cells cultured for 3 and 7 days after incubation with mCherry-CBD and mTagBFP-CBD. Bars, 5 μm.

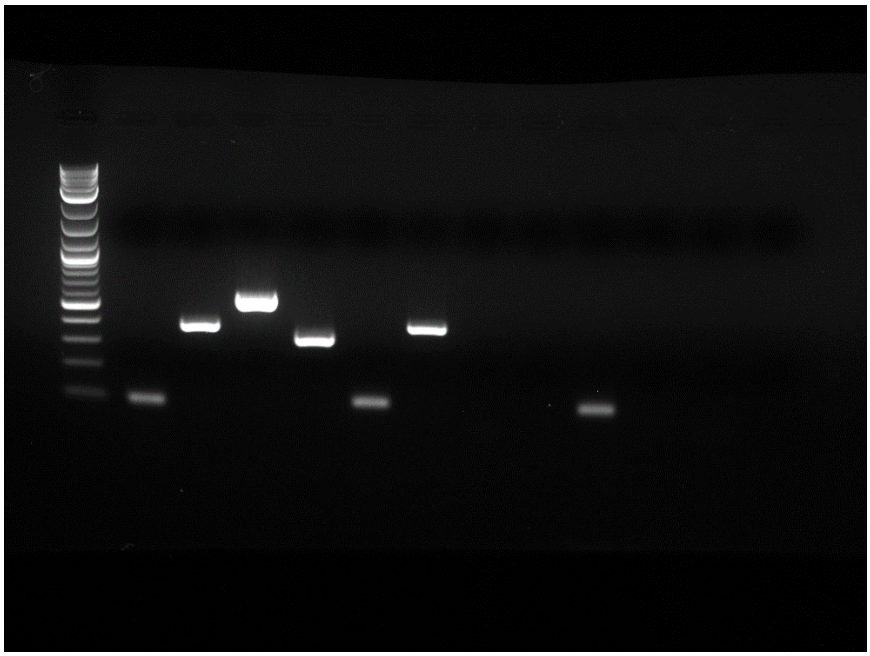


**Figure S6.** Original gel image used to prepare Figure 3C.

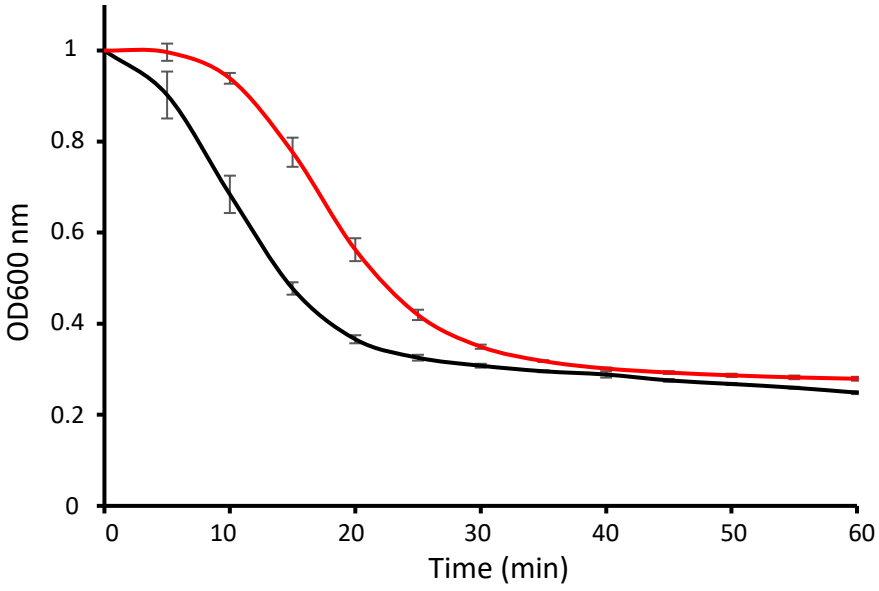



**Figure S7.** Turbidity reduction assay by measuring $OD_{600}$ of vegetative cells of *C. botulinum* Group I ATCC3502 (red line) and ATCC19397 (black line) over 60 min after addition of 5 µM of lysin CBO1751. All $OD_{600}$ values were normalized to an initial $OD_{600}$ of 1. Data are presented as means of three replicates ± standard deviations.