

SUPPLEMENTARY INFORMATION

Human commensal gut Proteobacteria withstand type VI secretion attacks through immunity protein-independent mechanisms

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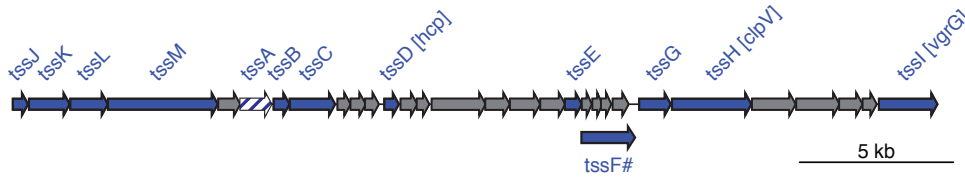
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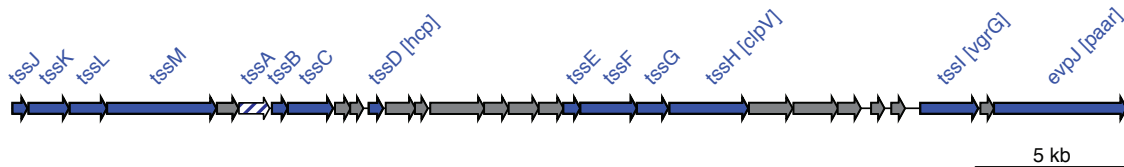
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Supplementary Figure S1

Commensal #10 (*E. cloacae*) T6SS-1



Commensal #11 (*E. ludwigii*) T6SS-1



Commensal #11 (*E. ludwigii*) T6SS-2

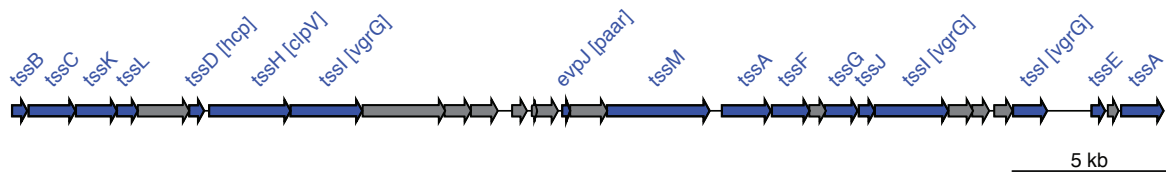


Fig. S1. Commensals belonging to the *Enterobacter cloacae* complex encode T6SS gene cluster(s). The genome sequences of the human commensal *Enterobacter* isolates #10 and #11 were inspected for the presence of T6SS gene cluster(s) using the program TXSScan. The core components of the T6SS detected with the program are indicated in blue. Manually annotated genes (*tssA*) are indicated by dashed arrows. #, indicates a region that was reassessed by Sanger sequencing, which revealed a sequencing artifact in the WGS data (details provided in Supplementary Data 5); the corrected non-interrupted *tssF* gene is depicted. Based on their gene organization, the clusters were classified as T6SS-1 or T6SS-2. Underlying source data are provided in the Source Data file and Supplementary Data files 4 and 5.

Supplementary Figure S2

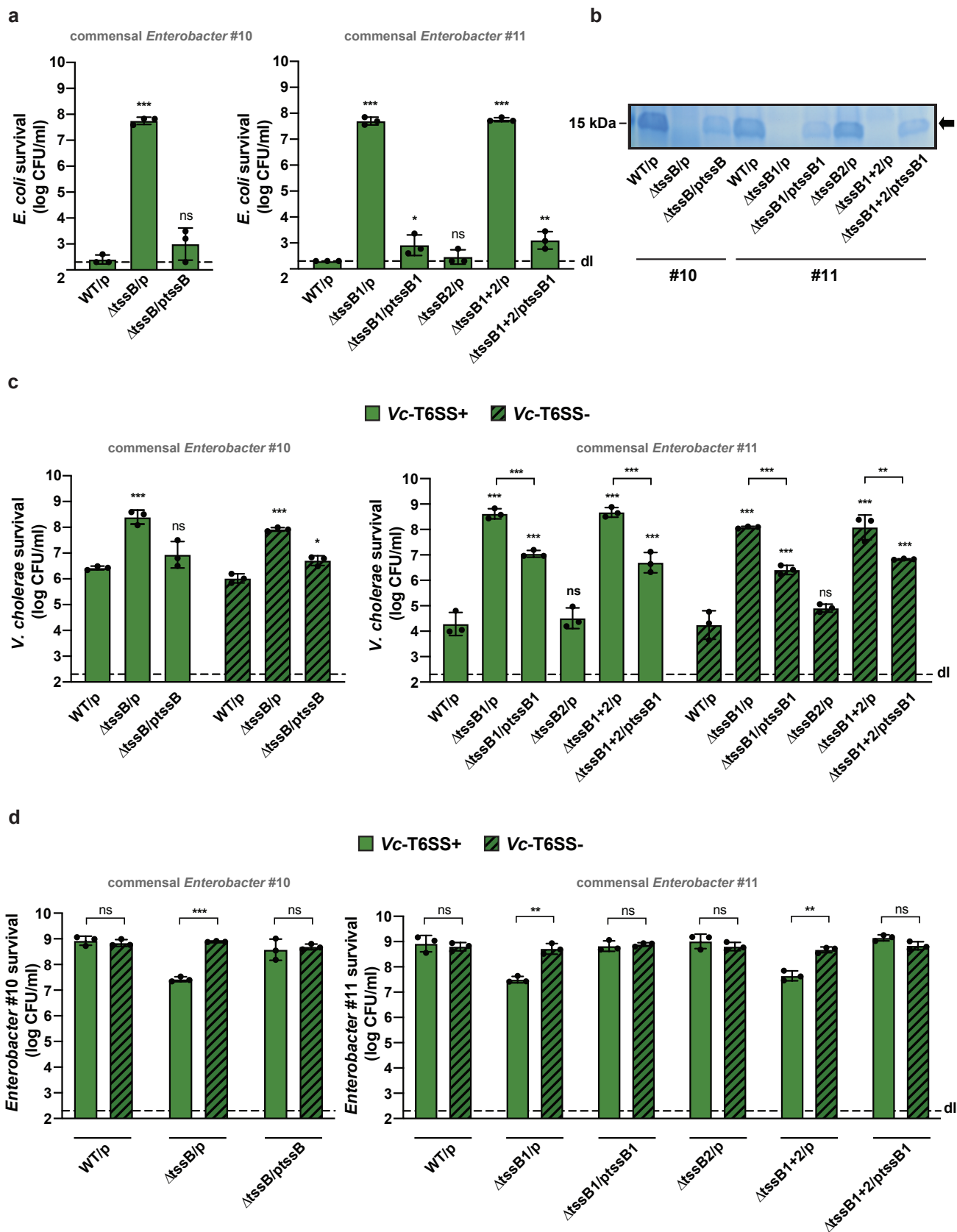
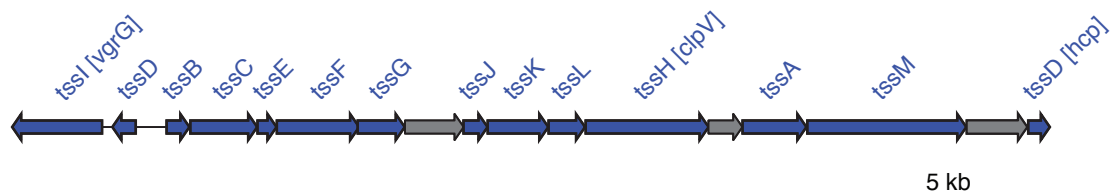


Fig. S2. *Enterobacter* complex isolates kill *E. coli* and compete with *V. cholerae* in a T6SS-1-dependent manner.

Survival of *E. coli* (a) or *V. cholerae* (c) was scored after coincubation with *Enterobacter* commensal strains #10 and #11 (carrying an empty plasmid [p], as control), their T6SS-1 and/or T6SS-2-negative mutants (Δ tssB for #10; Δ tssB1 and/or Δ tssB2 for #11; with empty plasmid [p]), or their T6SS-1-complemented derivatives (containing plasmids ptssB or ptssB1). Prey survival values are indicated on the Y-axis. (b) Detection of secreted Hcp. Details as for Fig. 2. Expression of the plasmid-encoded genes was induced with 0.2% arabinose. Representative image (out of three independent experiments). (d) *Enterobacter* survival after co-incubation with T6SS-positive (plain bar) and T6SS-negative (dashed bar) *V. cholerae*. (a, c, and d) Values are derived from three independent experiments and the bars represent the mean (\pm SD, as shown by the error bars). dl, detection limit, as indicated by the dashed line. Significant differences were determined using a one-way ANOVA followed by Holm-Sidak's multiple comparison test. Statistical significance indicated above each bar reflects comparison to the corresponding WT sample of each group (WT/p), except where comparison groups are explicitly shown by the brackets (a, c). For panel d, significant differences between samples containing T6SS+ and T6SS- predators were determined using a two-sided Student's *t*-test corrected for multiple comparisons. *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$; ns, not significant. Source data underlying all panels are provided in the Source Data file.

Supplementary Figure S3

Commensal #8 (*Klu. cryocrescens*) T6SS-3



Commensal #9 (*Klu. cryocrescens*) T6SS-3

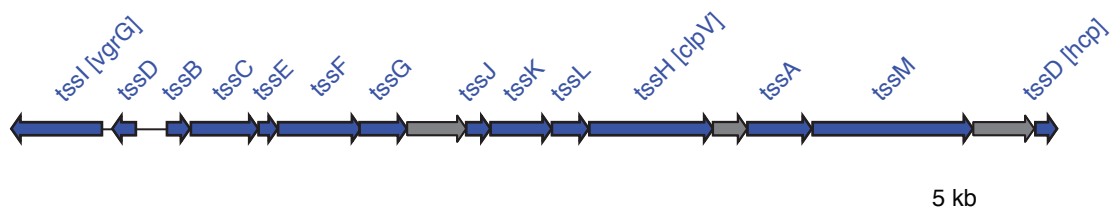


Fig. S3. Commensal *Kluyvera cryocrescens* strains harbor a T6SS-3 gene cluster. A third T6SS gene cluster (T6SS-3) with a different gene order compared to the T6SS-1- and T6SS-2 clusters was identified in the *Kluyvera cryocrescens* commensals #8 and #9. Underlying source data are provided in the Source Data file and Supplementary Data files 2 and 5.

Supplementary Figure S4

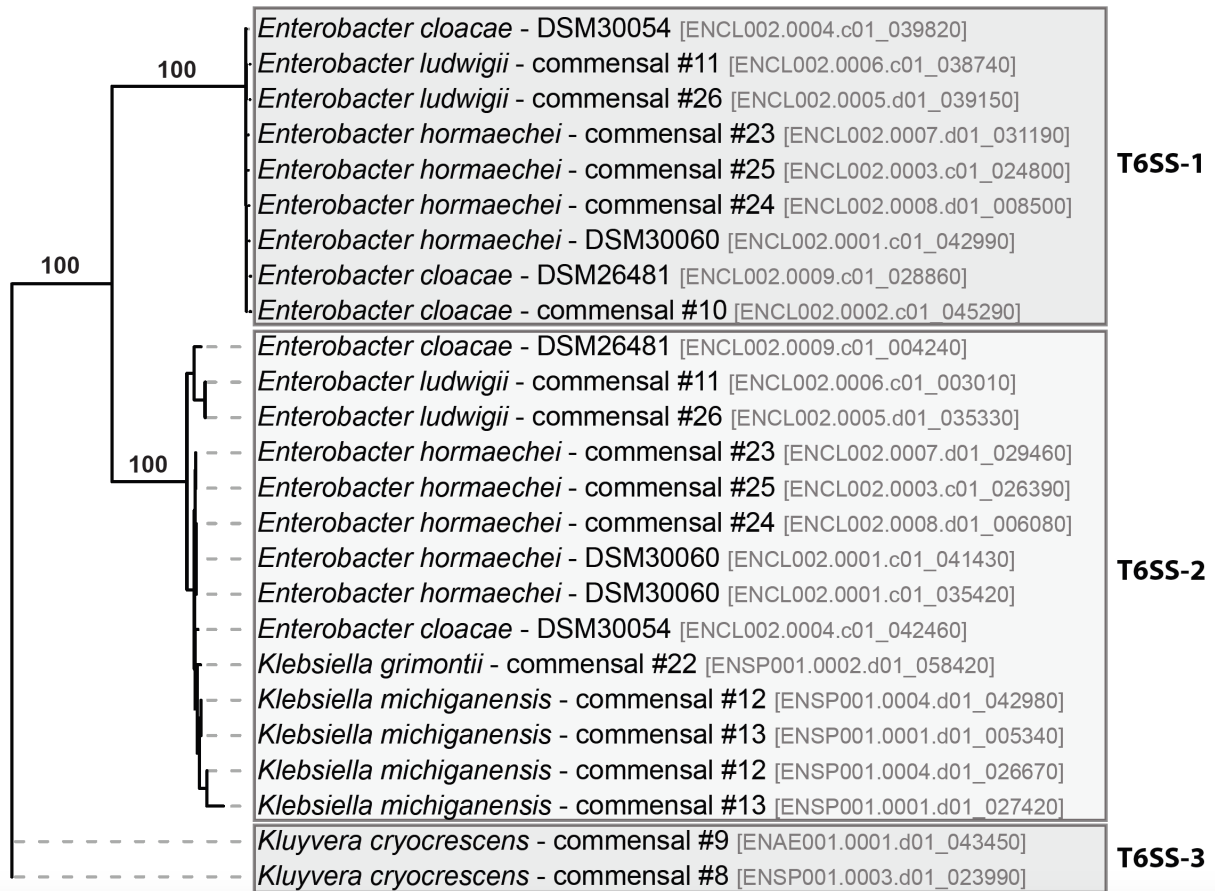
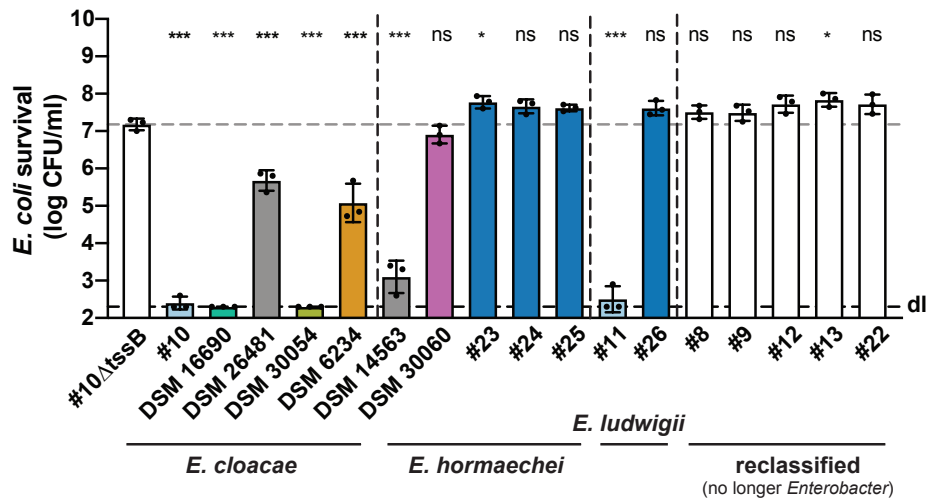


Fig. S4. TssB/TssC phylogenetic tree supports the presence of three distinctives T6SS clusters. A phylogenetic tree was constructed based on the concatenated alignments of TssB and TssC protein sequences from the T6SS clusters. Bootstrap values are indicated for the main branches. The ID names (in light gray) correspond to the individual TssB proteins (see Supplementary Data 5 and 7 for details). Underlying source data are provided in the Source Data file and Supplementary Data files 2, 4, and 5.

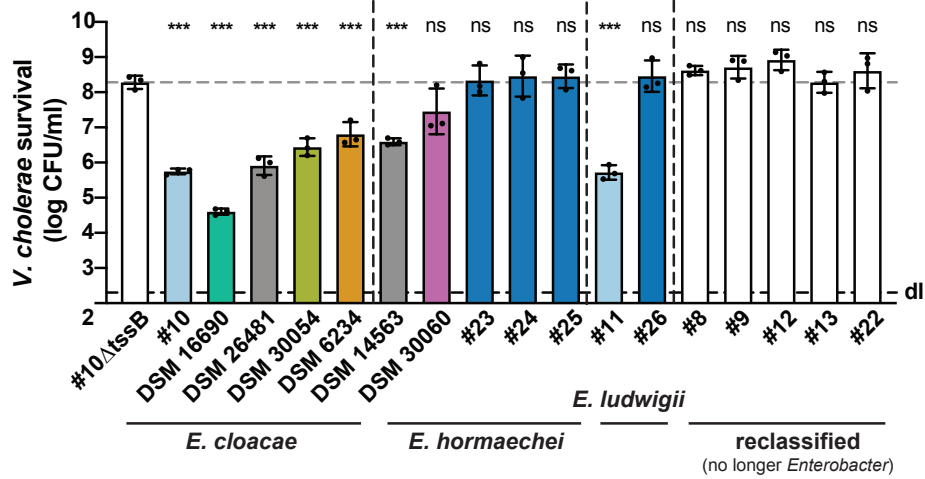
Supplementary Figure S5

a



- commensal (adult)
- commensal (baby)
- fecal isolate
- spinal fluid
- cleaning water of brewery
- minced meat
- unknown
- control or non-*Enterobacter*

b



c

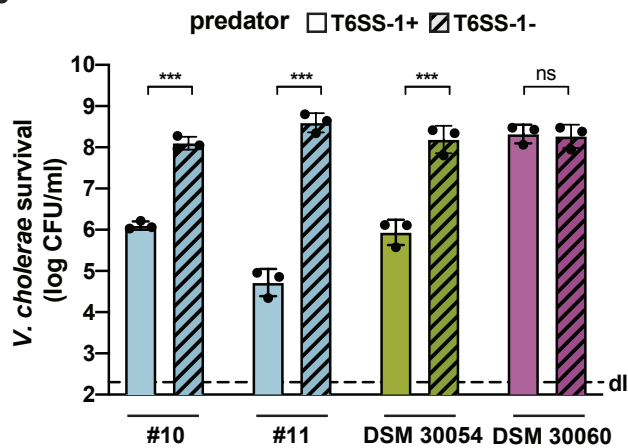
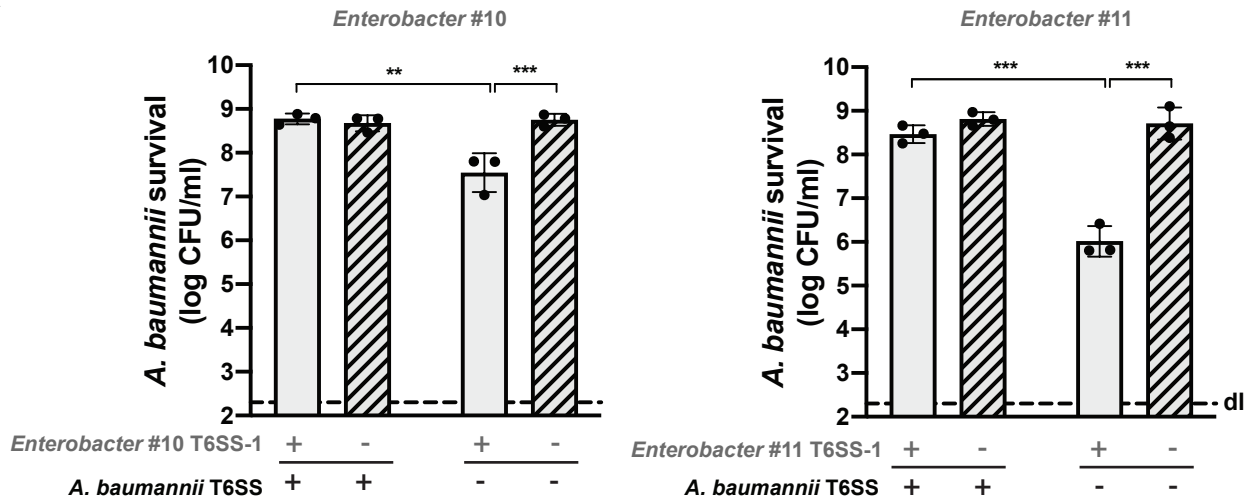


Fig. S5. A subset of *E. cloacae* complex strains kills *E. coli* and *V. cholerae* in a T6SS-1-dependent manner. Survival of *E. coli* (a) or *V. cholerae* (b, c) was scored after coincubation with a collection of *E. cloacae* complex strains (entire set compared to the selection shown in Fig. 2), including initially misclassified isolates (#8, #9, #12, #13, #22), and selected T6SS-1-negative derivatives (panel c). Values are derived from three independent experiments and the bars represent the mean (\pm SD, as shown by the error bars). dl, detection limit, as indicated by the black dashed line. Significant differences were determined using a one-way ANOVA followed by Holm-Sidak's multiple comparison test comparing each strain to the T6SS-deficient control commensal strain (#10 Δ tssB; value indicated by the dotted gray line) (a, b) or using a two-sided Student's *t*-test corrected for multiple comparison (c). *, $p < 0.05$, **, $p < 0.01$; ***, $p < 0.001$; ns, not significant. The colors reflect the origin of the isolates, as shown by the legend on the right of the figure. Source data underlying all panels are provided in the Source Data file.

Supplementary Figure S6

a



b

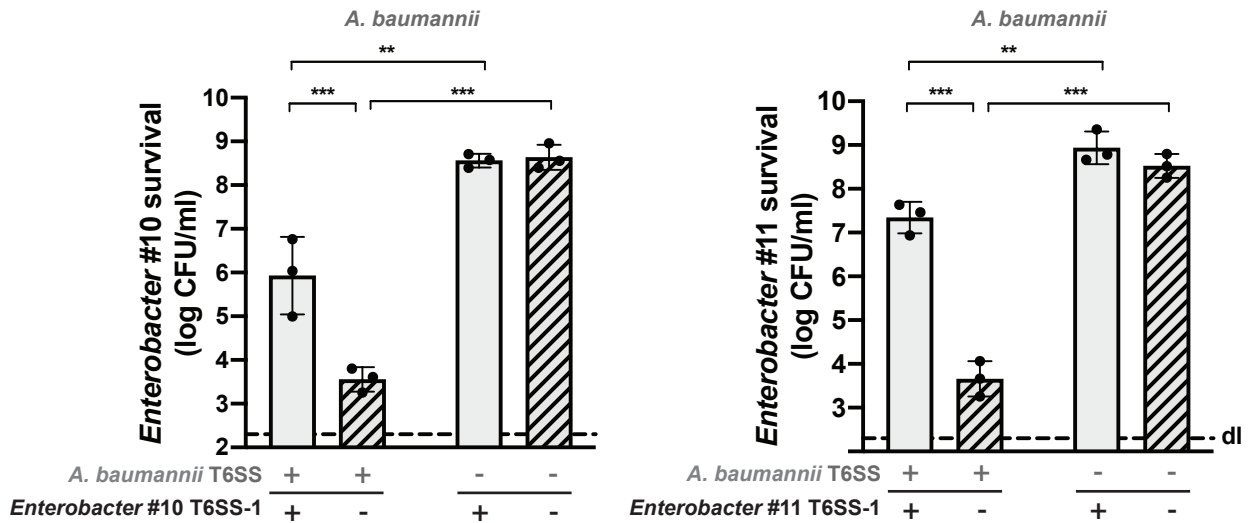
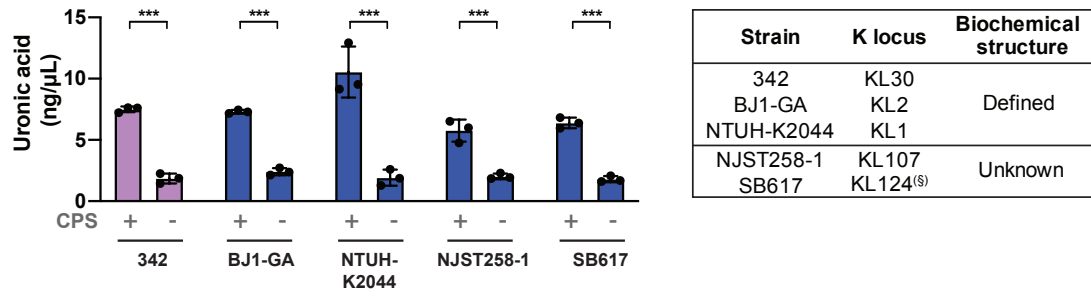


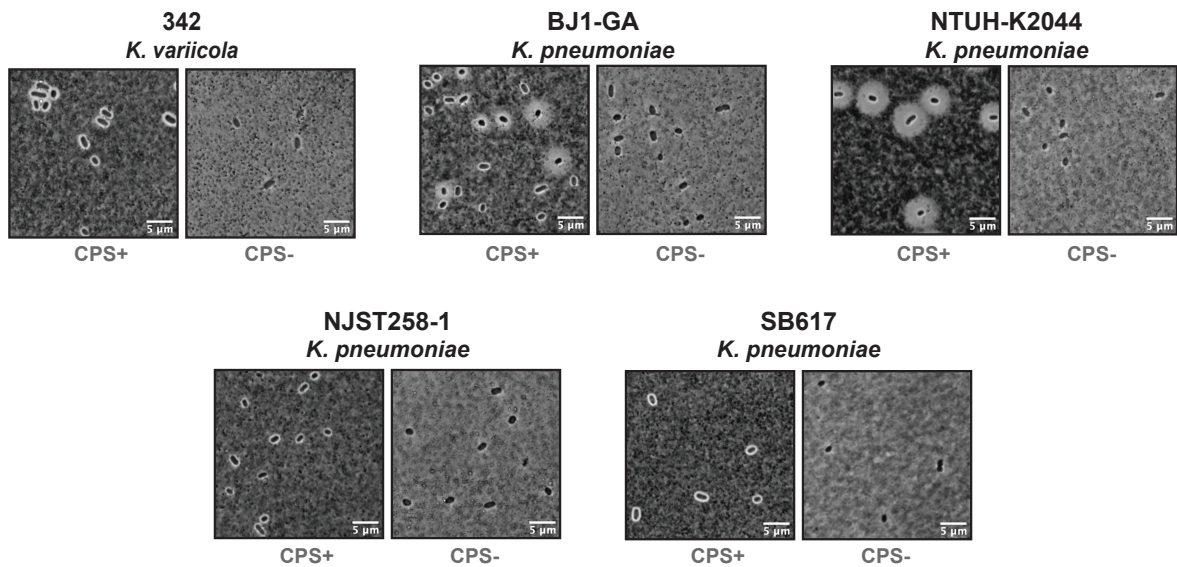
Fig. S6. *Acinetobacter baumannii* and commensal *Enterobacter* isolates compete in a T6SS-dependent manner. Wild-type (T6SS+) and T6SS-deficient (T6SS-) *A. baumannii* were co-incubated with WT *Enterobacter* isolates #10 and #11 or their T6SS-1-deficient mutants (#10 Δ tssB and #11 Δ tssB1). The survival of *A. baumannii* (a) and *Enterobacter* (b) was scored as indicated on the Y-axis. Values are derived from three independent experiments and the bars represent the mean (\pm SD, as defined by the error bars). Significant differences were determined using a two-sided Student's *t*-test corrected for multiple comparisons. Only statistically significant differences are shown. **, $p < 0.01$; ***, $p < 0.001$. Source data underlying all panels are provided in the Source Data file.

Supplementary Figure S7

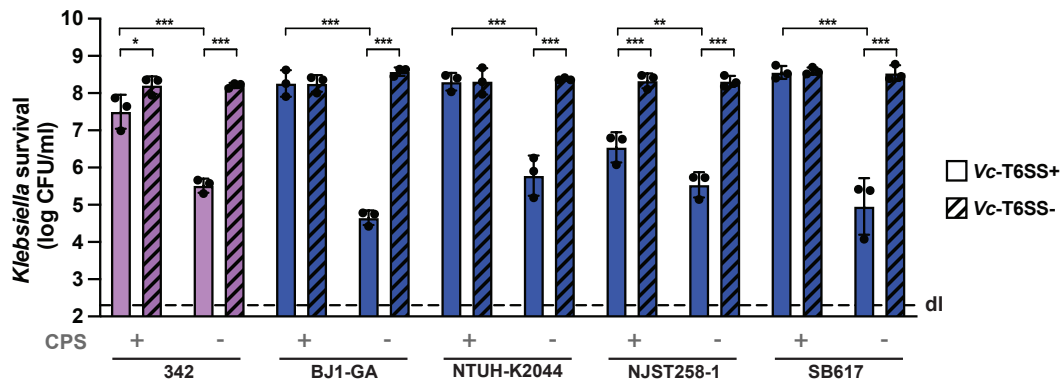
a



b



c



d

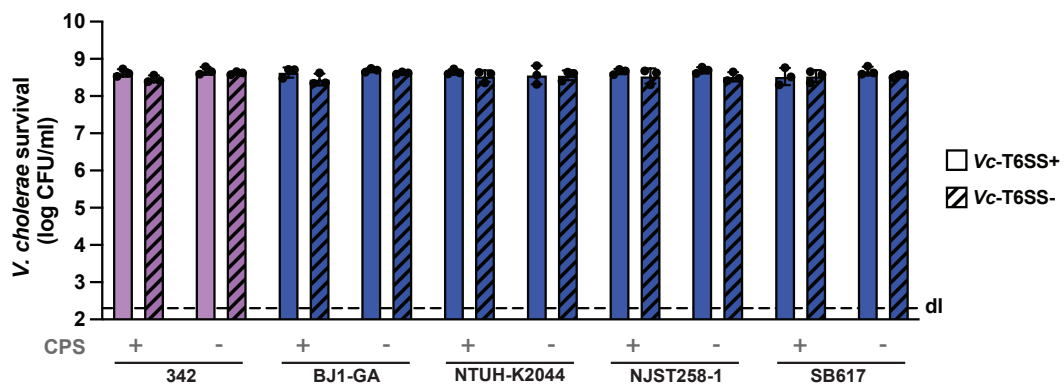
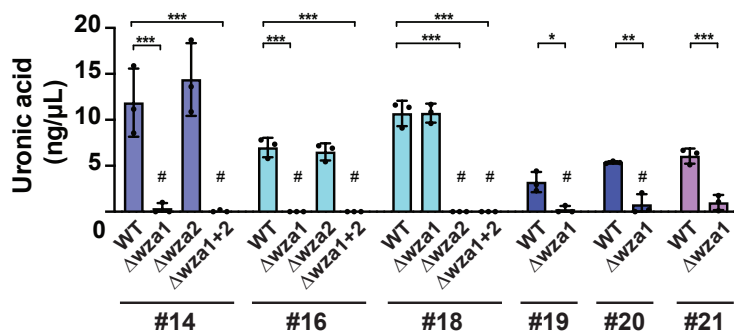


Fig. S7. Group I capsule protect *Klebsiella* species against *V. cholerae*'s T6SS attacks. (a) Deletion of the capsule biosynthesis gene *wza* eliminates capsule formation in well-studied *Klebsiella* strains with different K serotypes. Group I capsules were scored by uronic acid quantification in the CPS+ (*wza*-positive) and CPS- (*wza*-negative) bacteria. The table indicates the K antigen prediction by the Kaptive program. (§), low confidence prediction. (b) CPS+ and CPS- strains (Δwza) were imaged after India ink staining. Representative images are shown. Scale bar, 5 μ m. (c) Non-encapsulated strains are susceptible to *V. cholerae*'s T6SS assaults. The CPS+ and their polysaccharide export impaired CPS- *Klebsiella* strains were tested for survival against T6SS+ (WT; plain bars) or T6SS- ($\Delta vipA$; striped bars) *V. cholerae*, as indicated on the Y-axis. (d) *V. cholerae* is not killed by *Klebsiella* strains. T6SS+ (WT; plain bars) or T6SS- ($\Delta vipA$; striped bars) *V. cholerae* were tested for survival against CPS+ and their polysaccharide export impaired CPS- *Klebsiella* strains as indicated on the X-axis. Panels a, c and d: Values are derived from three independent experiments and the bars represent the mean (\pm SD, as defined by the error bars). Significant differences were determined using a two-sided Student's *t*-test corrected for multiple comparisons. Only significant differences within each *Klebsiella* strain group (as shown below the X-axis) are indicated. *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$. dl, detection limit, as indicated by the dashed line. Source data underlying all panels are provided in the Source Data file.

Supplementary Figure S8

a



b

Commensal	Species	Predicted K serotype	wza in capsule cluster
#14	<i>K. michiganensis</i>	-	wza1
#16	<i>K. oxytoca</i>	-	wza1
#18	<i>K. oxytoca</i>	-	wza2
#19	<i>K. pneumoniae</i>	KL31	wza1
#20	<i>K. pneumoniae</i>	KL31	wza1
#21	<i>K. variicola</i>	KL61	wza1

c

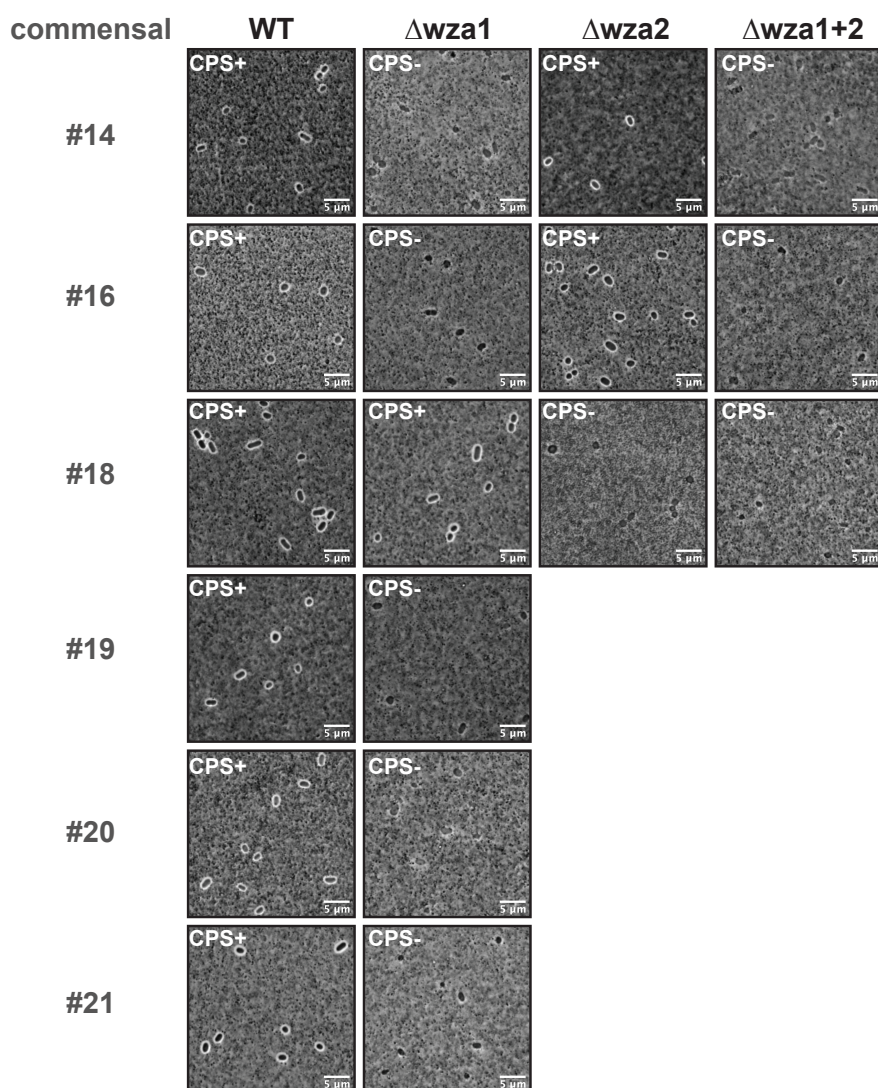
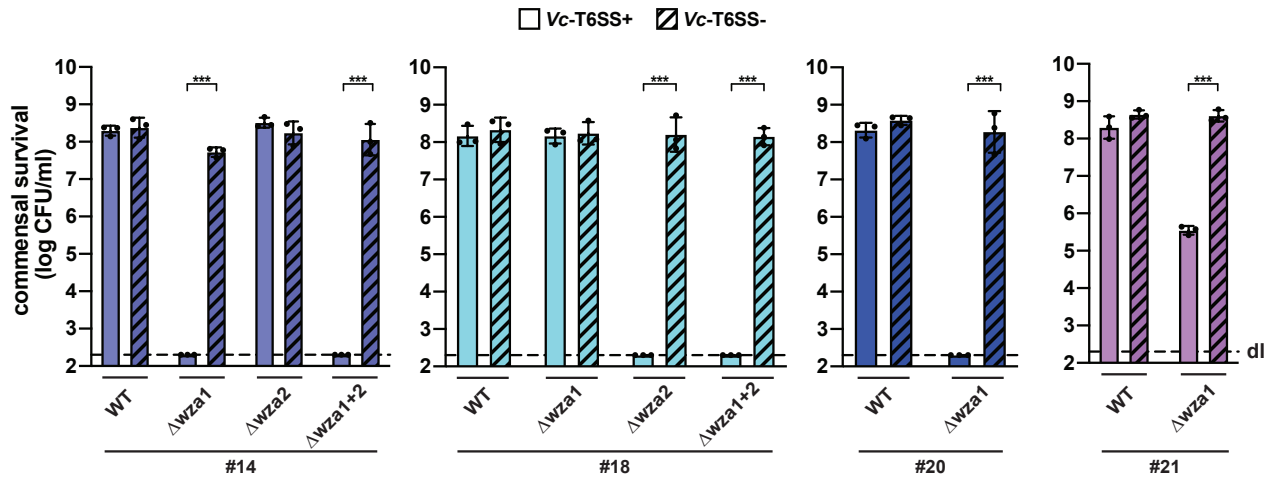


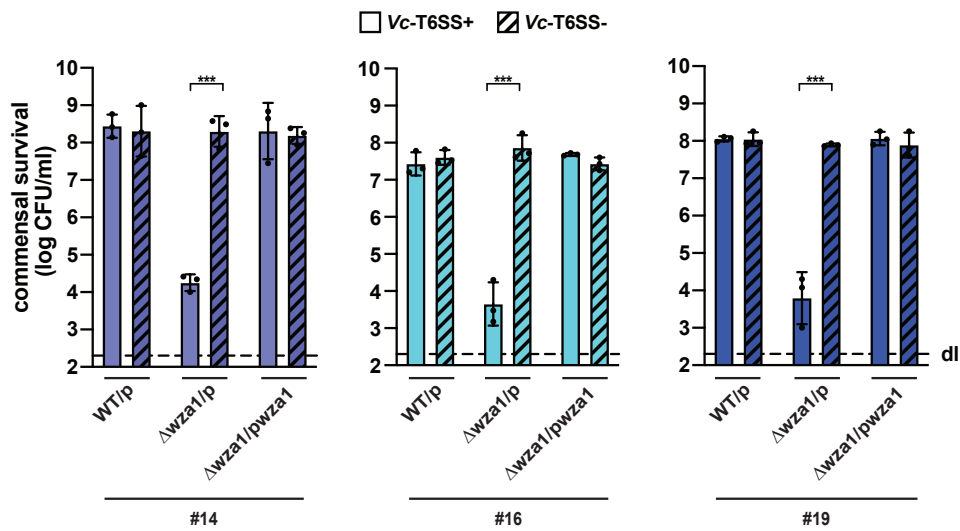
Fig. S8. Deletion of *wza* in the capsular biosynthesis gene cluster blocks capsule production in commensal *Klebsiella* strains. (a) The production of group I capsules by the *Klebsiella* gut commensal isolates was assessed by quantification of the strains' uronic acid content. The color code as well as the X-axis labels are as in Fig. 1. (b) Predicted K antigens of commensal *Klebsiella* isolates. The table indicates the *in silico* predicted K antigen (-, unpredictable) and which *wza* gene is located in the capsular operon. (c) Capsule visualization of *Klebsiella* WT (CPS+) and *wza*-minus strains after India Ink staining. Representative images are shown. Scale bar, 5 μ m. For panel a, values are derived from three independent experiments and the bars represent the mean (\pm SD, as defined by the error bars). #, at least one sample was below the detection limit. Significant differences were determined using a two-sided Student's *t*-test corrected for multiple comparisons. Only significant differences compared to the corresponding WT strain are indicated. *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$. Source data underlying all panels are provided in the Source Data file.

Supplementary Figure S9

a



b



c

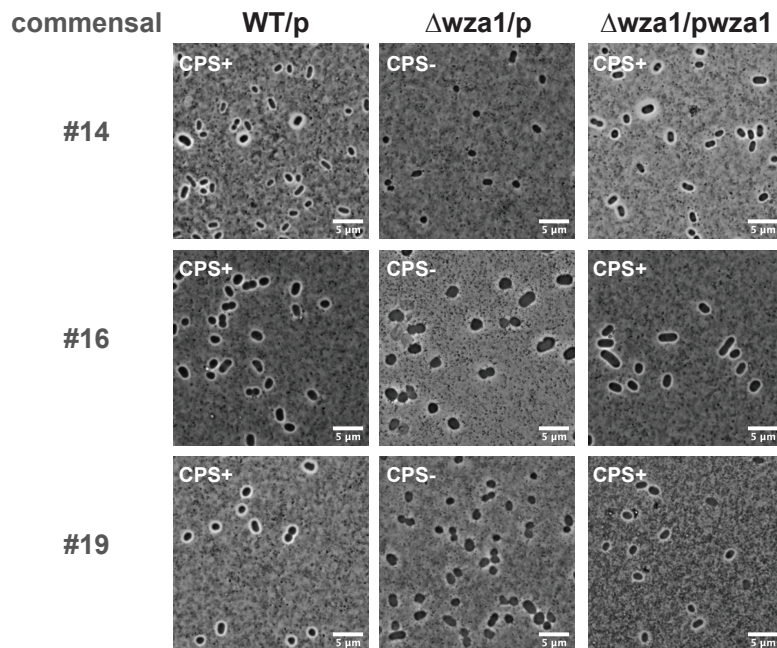


Fig. S9. Group I capsule protects against T6SS attacks from *V. cholerae*. (a) Extended set of representative WT and polysaccharide export protein-negative mutants ($\Delta wza1$ and/or $\Delta wza2$) of *Klebsiella* commensal strains (#14, #18, #20, #21) showing their survival after coincubation with T6SS+ (plain bars) or T6SS- ($\Delta vipA$; stripped bars) *V. cholerae* (as for #16 and #19 in Fig. 4c). (b and c) Complementation of capsule-deficient mutant strains. The capsule-deficient mutants of the commensal *Klebsiella* strains #14, #16, #19 were complemented with their respective *wza1* gene copy on a plasmid *in trans* (pwza1). The complemented strains as well as the vector controls of the WT and mutant strains (containing the empty plasmid [p]) were tested for their survival in the presence of T6SS+ (WT; plain bars) or T6SS- ($\Delta vipA$; stripped bars) *V. cholerae* (panel b) or imaged after Indian Ink staining to check their capsulation status (panel c). Scale bar, 5 μ m. dl, detection limit, as indicated by the dashed line. Panels a-b: Values are derived from three independent experiments and the bars represent the mean (\pm SD, as defined by the error bars). Significant differences were determined using a two-sided Student's *t*-test corrected for multiple comparisons. Only significant differences are indicated. ***, $p < 0.001$. Source data underlying all panels are provided in the Source Data file.

Supplementary Figure S10

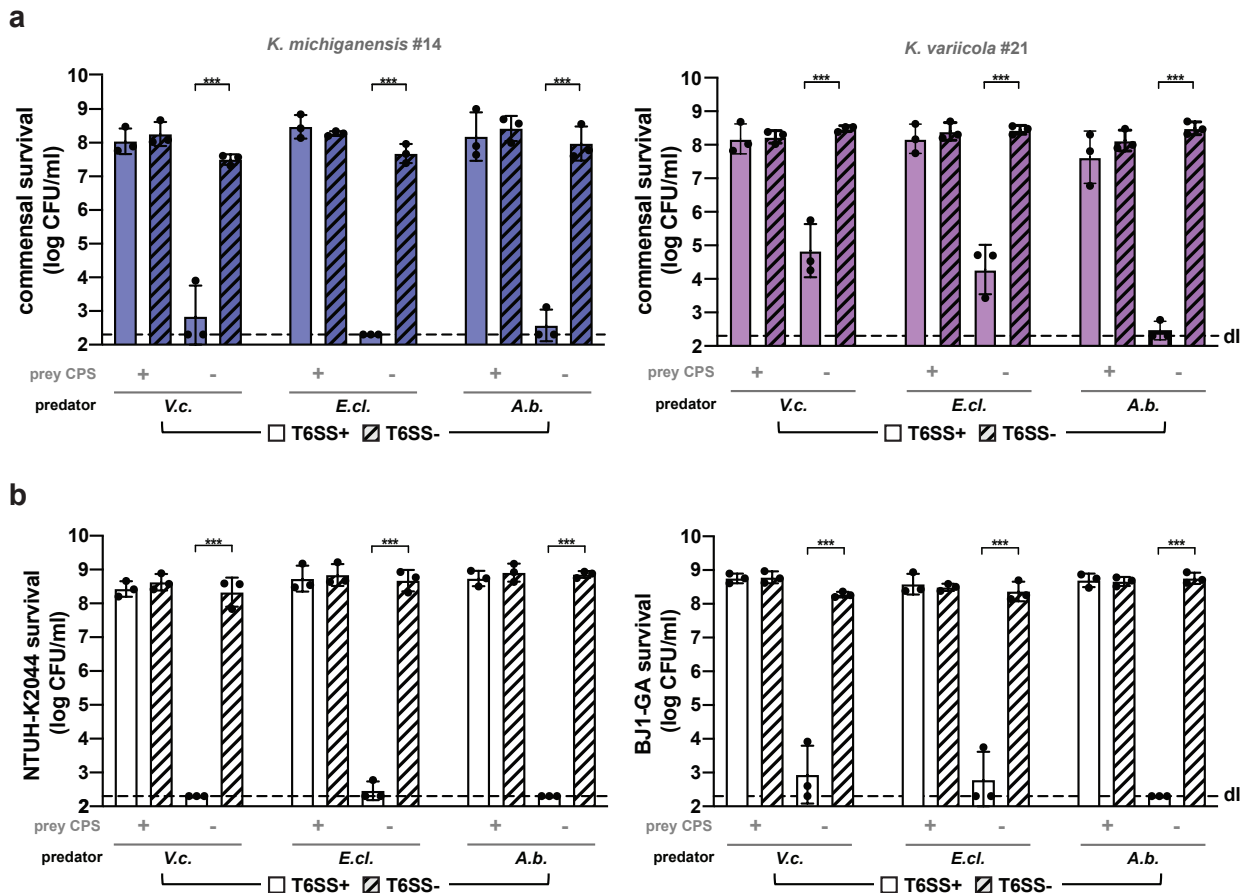


Fig. S10. Group I capsule protects *Klebsiella* species against T6SS attacks from pathogenic bacteria.

Additional examples of commensal *Klebsiella* WT (*K. michiganensis* #14 and *K. variicola* #21) and *wza*-negative mutants (a) or the well-studied CPS-positive (CPS+) *K. pneumoniae* strains NTUH-K2044 and BJ1-GA and their CPS-negative (CPS-) mutants (b) were tested for their survival in the presence of diverse T6SS+ (WT; plain bars) or T6SS- (striped bars) pathogenic bacteria (*V. cholerae* [*V.c.*], *E. cloacae* [*E.cl.*; commensal #10], or *A. baumannii* [*A.b.*; strain A118]). Values are derived from three independent experiments and the bars represent the mean (\pm SD, as defined by the error bars). dl, detection limit, as indicated by the dashed line. Only significant differences are indicated. Significance was determined using a Student's *t*-test corrected for multiple comparisons. ***, $p < 0.001$. Source data underlying all panels are provided in the Source Data file.

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