FIGURE S1

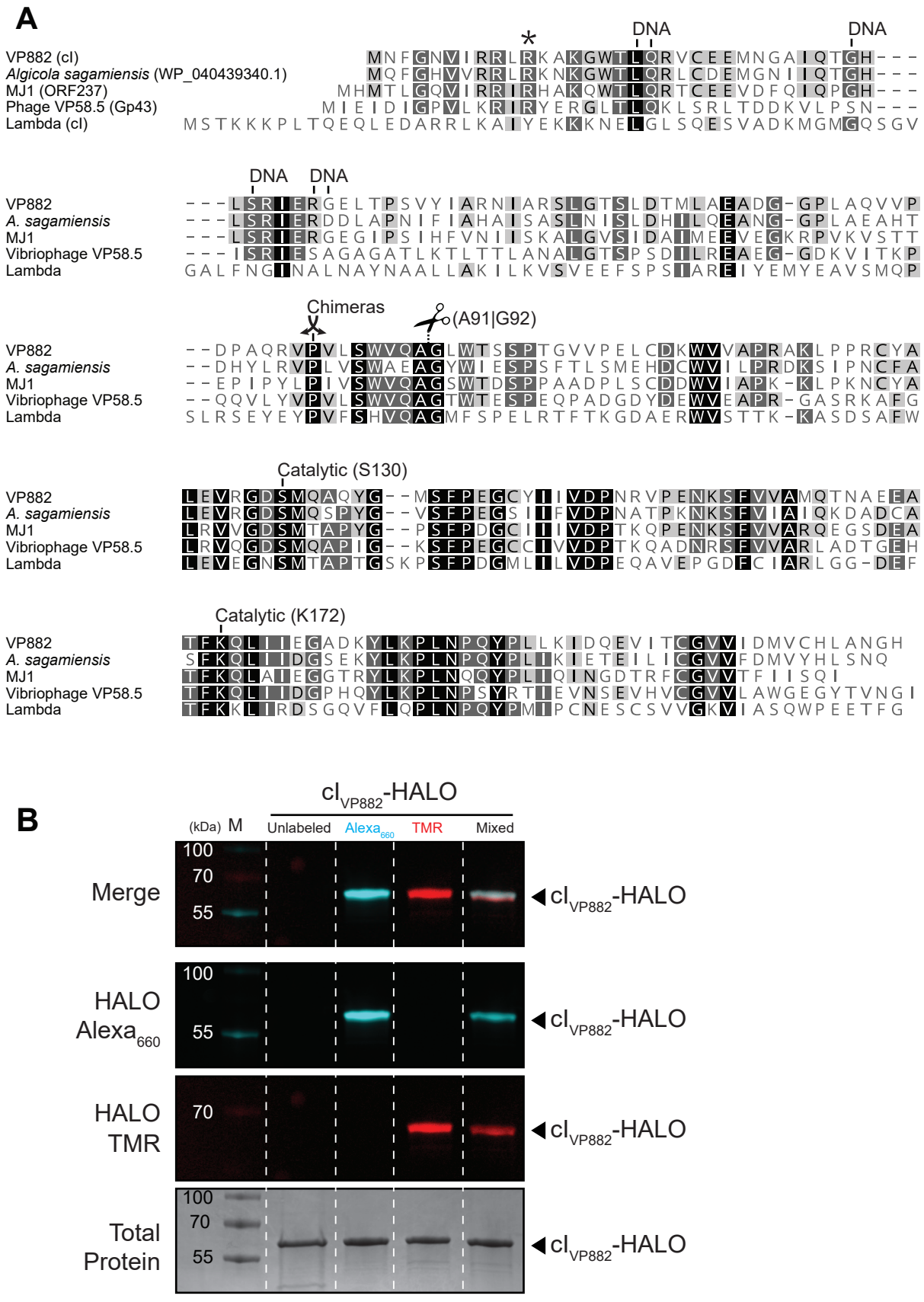

A
DNA
DNA
VP882 (cI)
Algicola sagamiensis (WP_040439340.1)
MJ1 (ORF237)
Phage VP58.5 (Gp43)
Lambda (cI)
DNA
DNA
VP882
A. sagamiensis
MJ1
Vibriophage VP58.5
Lambda
Chimeras
(A91|G92)
VP882
A. sagamiensis
MJ1
Vibriophage VP58.5
Lambda
Catalytic (S130)
VP882
A. sagamiensis
MJ1
Vibriophage VP58.5
Lambda
Catalytic (K172)
VP882
A. sagamiensis
MJ1
Vibriophage VP58.5
Lambda
B
cI VP882 -HALO
(kDa) M
Unlabeled
Alexa 660
TMR
Mixed
Merge
HALO Alexa 660
HALO TMR
Total Protein
100
70
55
cI VP882 -HALO

Figure S1. Sequence alignment of cI_{VP882} and other cI-type repressors, and differential HALO-tag labeling to detect proteins by SDS-PAGE analysis, Related to Figures 1, 2, and 3.

(A) Multiple sequence alignment of cI_{VP882} with three related cI-type repressors and cI_{Lambda}. Residues in cI_{VP882} predicted to participate in DNA binding are designated "DNA"; putative catalytic-sites are designated "Catalytic", the cleavage site, between a conserved alanine and glycine residue, is represented with scissors (see Figure 2C), sites in cI_{VP882} and cI_{Lambda} used to fuse domains for chimera construction are represented with crossed-arrows (see Figure 3B), and a site required for Qtip recognition is represented with an asterisk (see Figure 3C). Box shading indicates residues that are identical across all (black), the same in 4 of 5 (dark gray), or in 3 of 5 (light gray) proteins. (B) SDS-PAGE analysis of unlabeled cI_{VP882}-HALO, cI_{VP882}-HALO conjugated to HALO-$Alexa_{660}$ (cyan), cI_{VP882}-HALO conjugated to HALO-TMR (red), or when the separately labeled proteins were combined. $Alexa_{660}$ but not TMR-labeled proteins are detected with the Cy5 filter set, designated HALO-$Alexa_{660}$. TMR- but not $Alexa_{660}$- labeled proteins are detected with the Cy3 filter set, designated HALO-TMR. The composite of the HALO-$Alexa_{660}$ and HALO-TMR channels is shown in the upper-most panel, designated Merge. Unlabeled cI_{VP882}-HALO is not detected using Cy3 or Cy5 filter sets. All proteins can be visualized by staining the gel for total protein with Coomassie Brilliant Blue, designated Total Protein. Molecular weight marker is designated M.

FIGURE S2

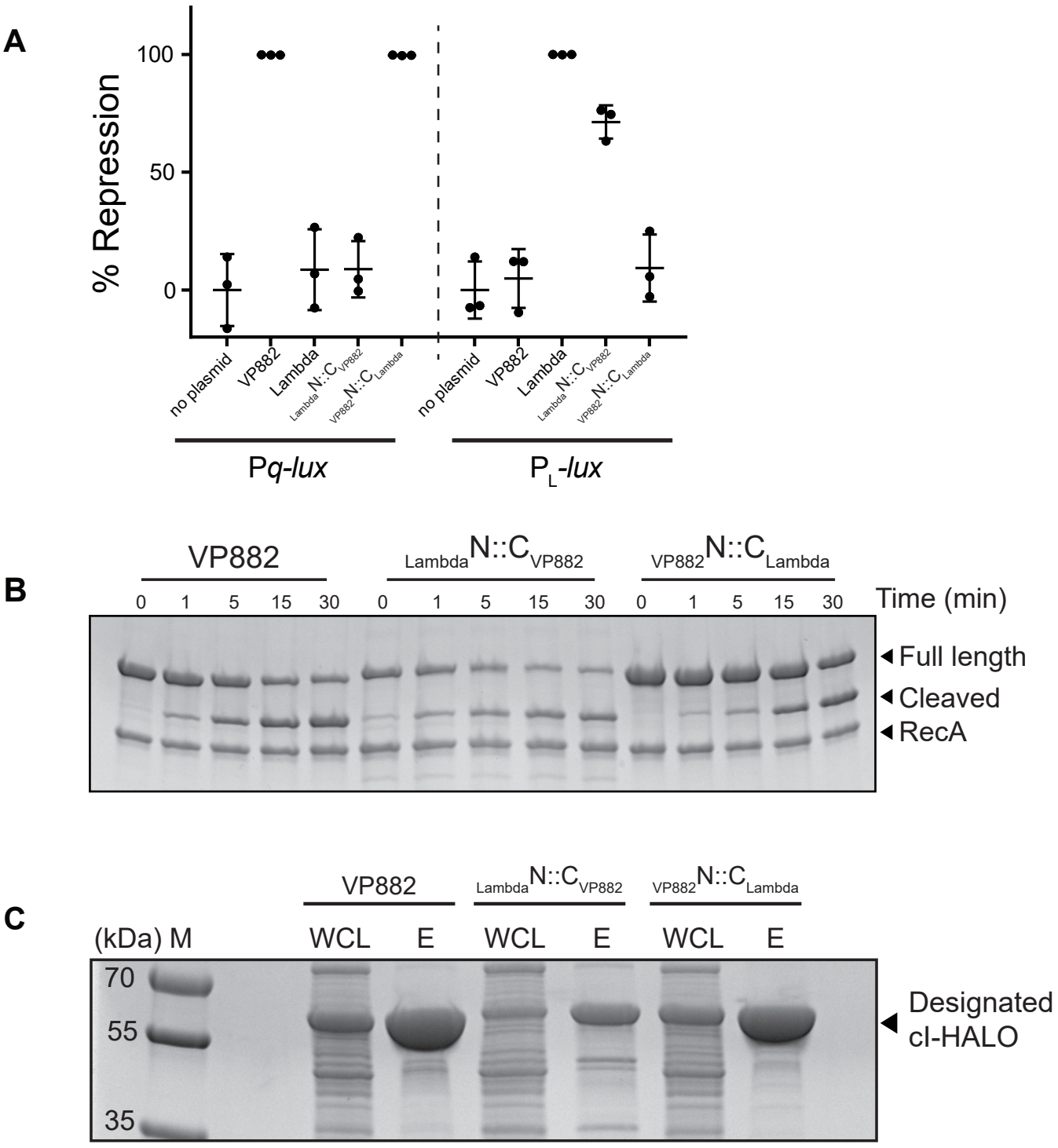

A
% Repression
100
50
0
no plasmid
VP882
Lambda
Lambda N::C VP882
VP882 N::C Lambda
Pq-lux
P L -lux
B
VP882
Lambda N::C VP882
VP882 N::C Lambda
0 1 5 15 30
Time (min)
Full length
Cleaved
RecA
C
(kDa) M
WCL
E
70
55
35
Designated cI-HALO

Figure S2. Chimeric cI repressors bind DNA and undergo cleavage *in vitro*, Related to Figure 3

(A) Repression of reporter expression by full-length and chimeric cI repressors. Left side, percent repression of the VP882-encoded P*q*-*lux* reporter or, right side, lambda-encoded P_L-*lux* reporter in *E. coli* lacking (no plasmid) or containing the designated cI repressor. %Repression is the difference in RLU obtained for each construct compared to the no-plasmid control. Data represented as mean ± SD with n = 3 biological replicates. (B) *In vitro* cleavage of cI_{VP882}-HALO and the chimeric fusions conjugated to HALO-TMR, monitored by SDS-PAGE analysis. Incubation times are noted above each lane. (C) SDS-PAGE analysis of whole-cell lysates (designated WCL) or purified proteins (designated E for eluate) used in (B). Note the lower yield and the presence of possible cleavage products of $_{Lambda}N::C_{VP882}$-HALO indicating possible protein instability. Molecular weight marker is designated M.

FIGURE S3

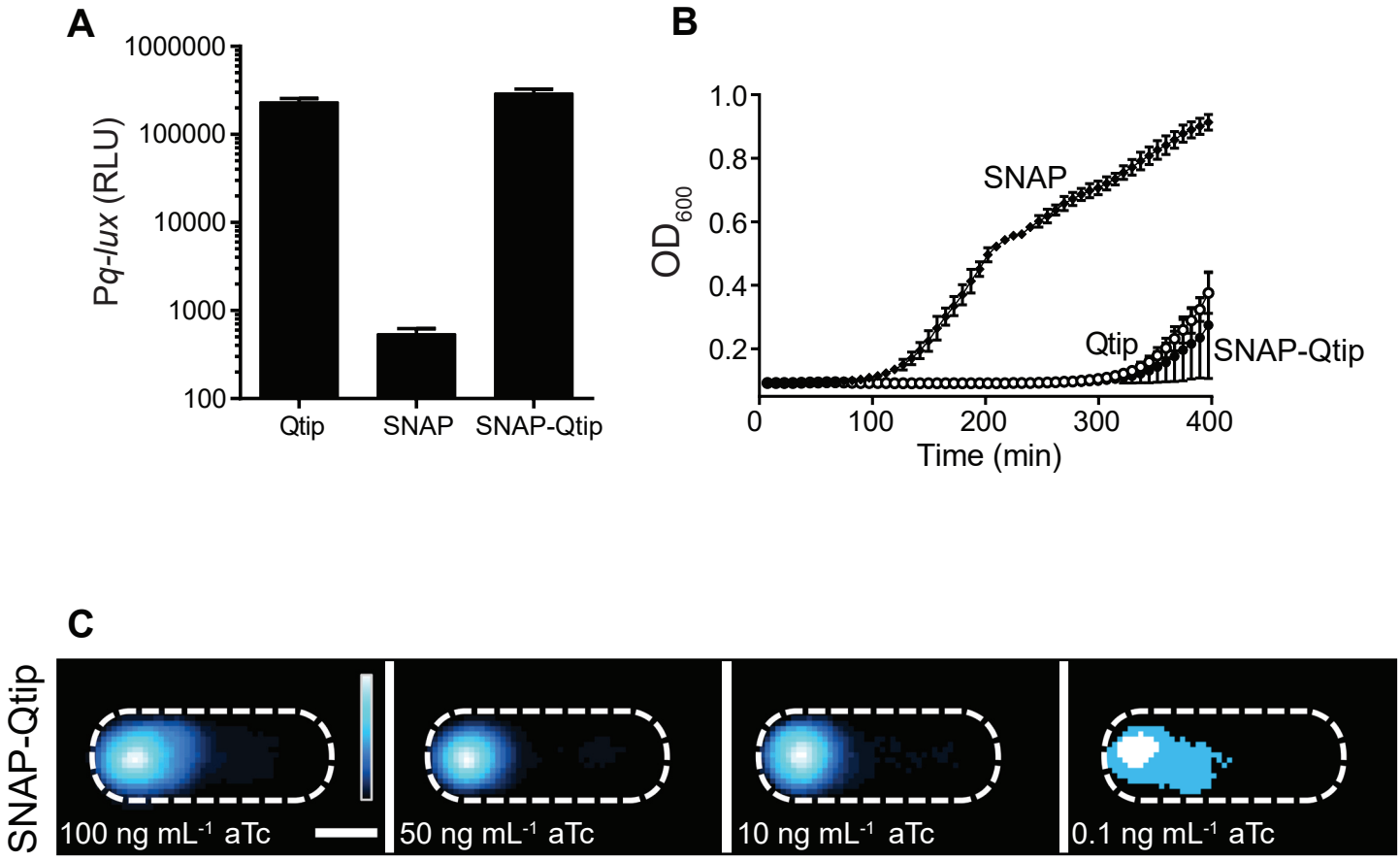
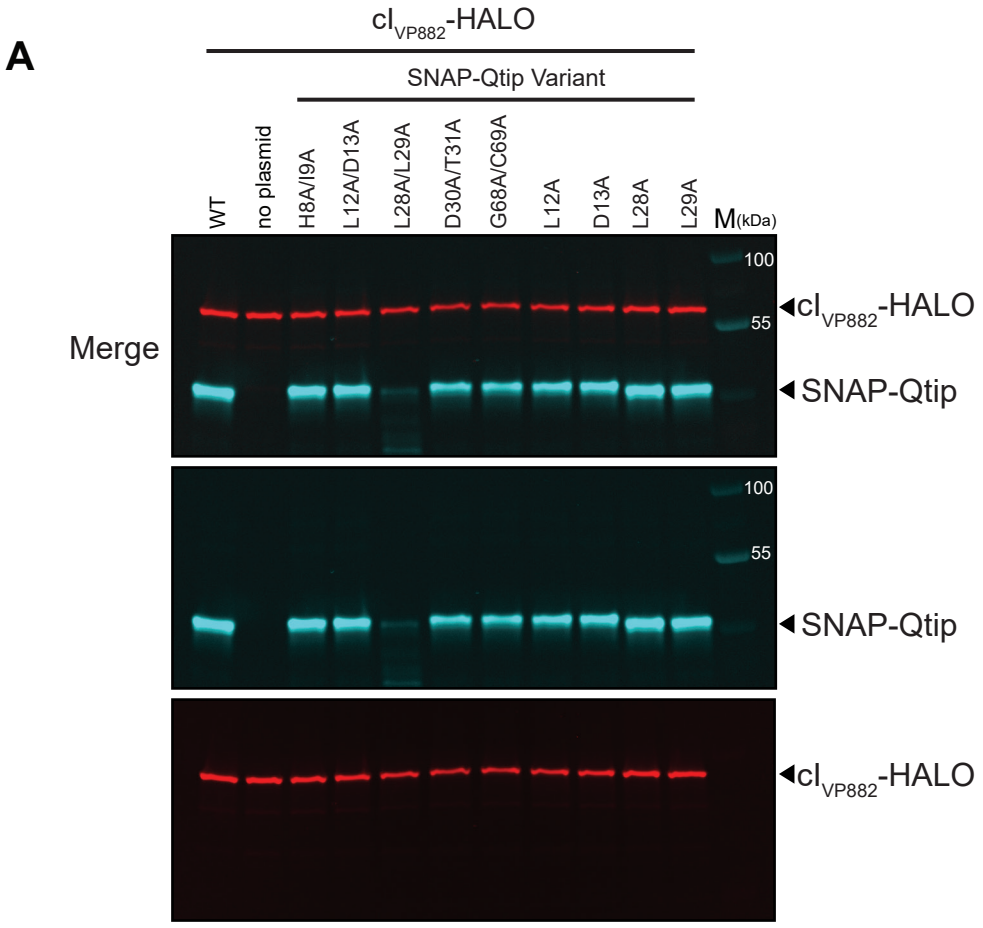

A
1000000
100000
10000
1000
100
Pq-lux (RLU)
Qtip
SNAP
SNAP-Qtip
B
1.0
0.8
0.6
0.4
0.2
OD600
SNAP
Qtip
SNAP-Qtip
0
100
200
300
400
Time (min)
C
SNAP-Qtip
100 ng mL-1 aTc
50 ng mL-1 aTc
10 ng mL-1 aTc
0.1 ng mL-1 aTc

Figure S3. The SNAP tag does not interfere with Qtip function and SNAP-Qtip localizes at the cell poles independent of the concentration of inducer, Related to Figure 4.

(A) Light production from the P*q-lux* reporter in *E. coli* producing cI_{VP882} and WT Qtip, SNAP, or SNAP-Qtip. (B) Growth curve of a *Vibrio parahaemolyticus* VP882 lysogen producing the same constructs from (A); SNAP (diamonds), Qtip (open circles), and SNAP-Qtip (closed circles). Data in (A) and (B) represented as mean ± SD with n = 3 biological replicates. (C) Composite images from individual cell analyses of *E. coli* harboring aTc-inducible SNAP-Qtip induced with the indicated amount of aTc. Samples labeled with SNAP-JF_{503} and displayed as in Figure 4A. As noted in the Methods, all images are internally contrasted. The pixelated appearance of the SNAP in the panel showing the 0.1 ng mL^{-1} aTc concentration is a consequence of low induction of SNAP fluorescence. Scale bar, as in Figure 1D.

FIGURE S4


A
cl$_{VP882}$-HALO
SNAP-Qtip Variant
WT
no plasmid
H8A/I9A
L12A/D13A
L28A/L29A
D30A/T31A
G68A/C69A
L12A
D13A
L28A
L29A
M(kDa)
100
55
Merge
cl$_{VP882}$-HALO
SNAP-Qtip
100
55
SNAP-Qtip
cl$_{VP882}$-HALO

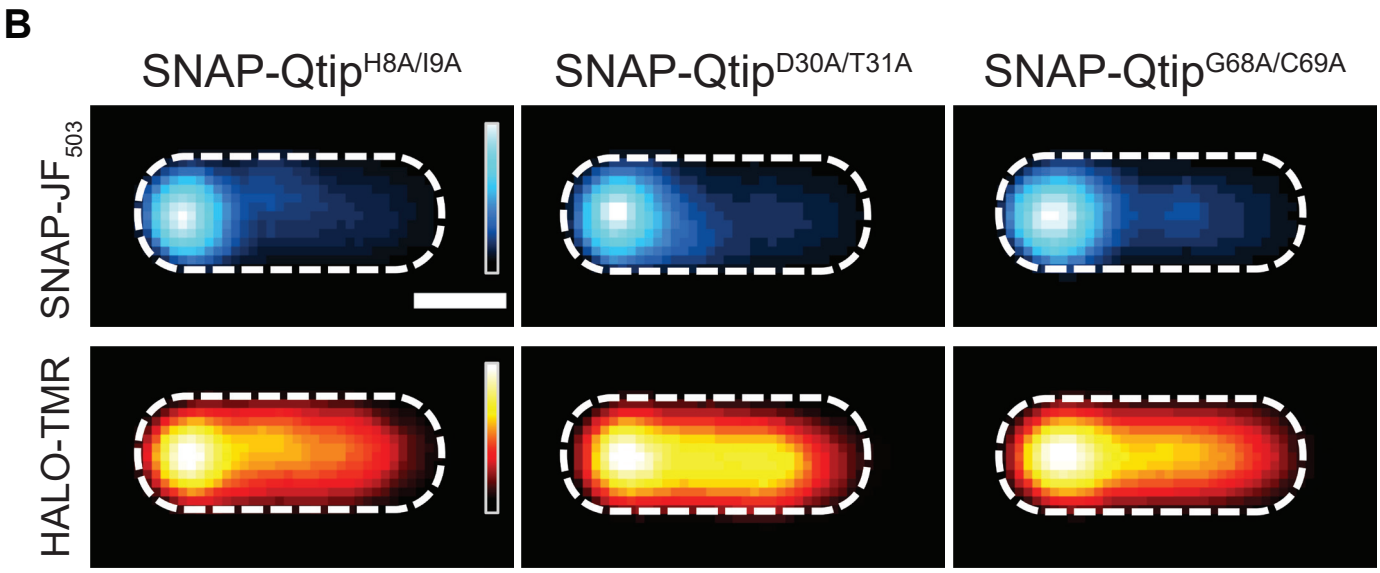

B
SNAP-Qtip$^{H8A/I9A}$
SNAP-Qtip$^{D30A/T31A}$
SNAP-Qtip$^{G68A/C69A}$
SNAP-JF$_{503}$
HALO-TMR

Figure S4. Production of WT SNAP-Qtip and SNAP-Qtip variants, and microscopic analysis of SNAP-Qtip$^{H8A/I9A}$, SNAP-Qtip$^{D30A/T31A}$, and SNAP-Qtip$^{G68A/C69A}$, Related to Figures 4 and 5.

(A) Multi-label SDS-PAGE analysis of *E. coli* harboring one plasmid encoding cI$_{VP882}$-HALO and either a second plasmid carrying WT SNAP-Qtip (leftmost lane), no plasmid (second lane) or the indicated SNAP-Qtip variant. SNAP-Qtip was labeled with the far-red fluorescent dye SNAP-Cell 647-SiR and cI$_{VP882}$-HALO was labeled with HALO-TMR. SNAP-Cell 647-SiR (cyan channel) and HALO-TMR (red channel) signals were visualized by imaging the gel under the Cy5 and Cy3 filter sets, respectively, followed by overlaying the channels into a single image (Merge). Molecular weight marker is designated M. (B) Composite images from individual cell analysis of *E. coli* producing cI$_{VP882}$-HALO and either SNAP-Qtip$^{H8A/I9A}$, SNAP-Qtip$^{D30A/T31A}$, or SNAP-Qtip$^{G68A/C69A}$. Samples labeled with SNAP-JF$_{503}$ and HALO-TMR, and displayed as in Figure 5B. Scale bar, as in Figure 1D.

FIGURE S5

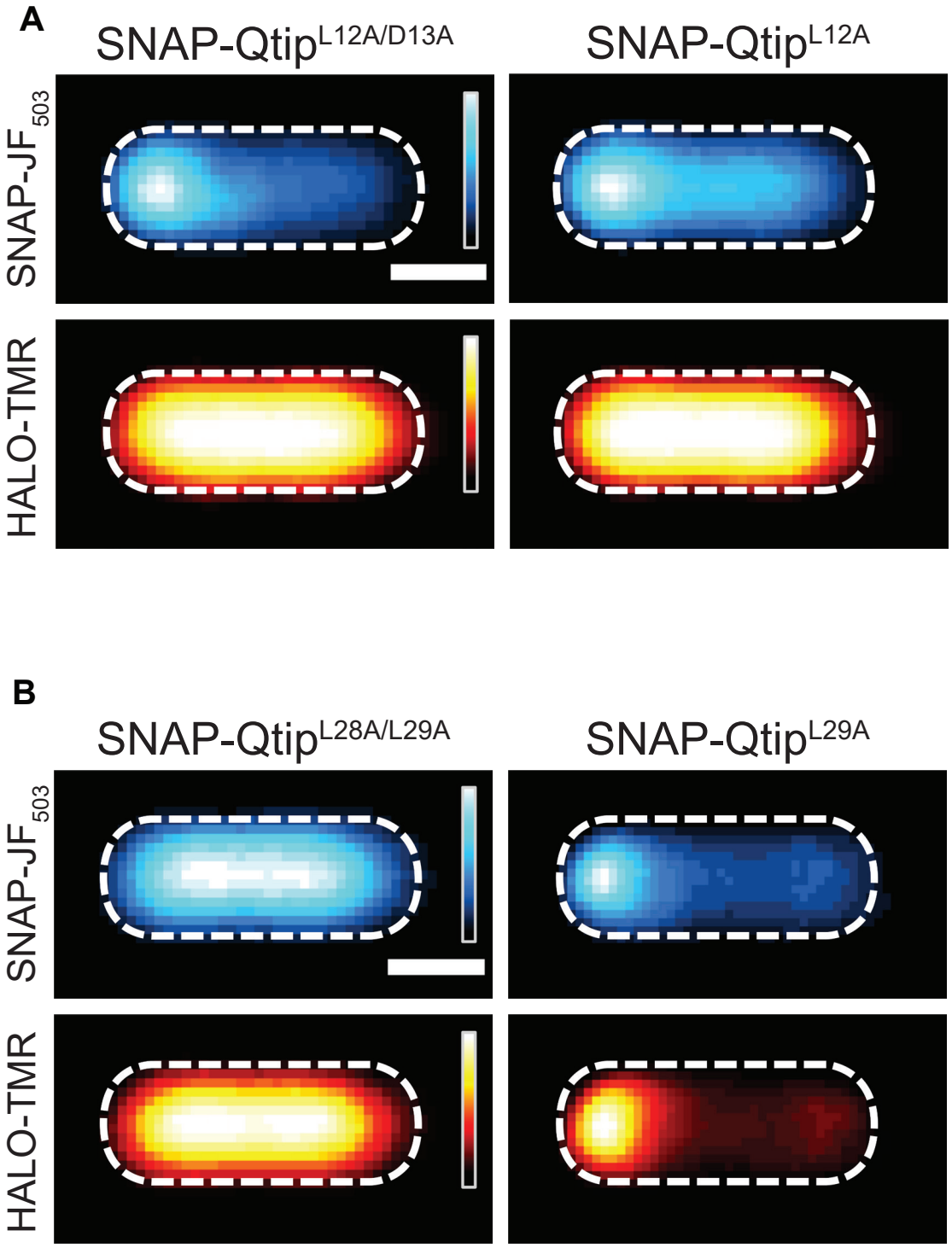
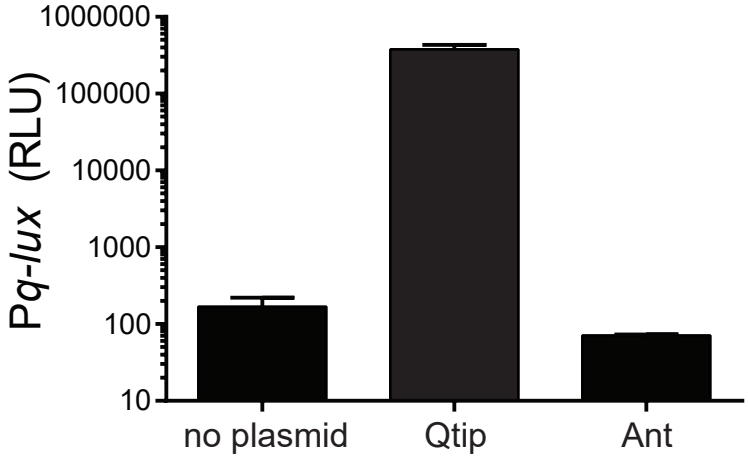

A
SNAP-Qtip$^{L12A/D13A}$
SNAP-Qtip^{L12A}
SNAP-JF$_{503}$
HALO-TMR
B
SNAP-Qtip$^{L28A/L29A}$
SNAP-Qtip^{L29A}
SNAP-JF$_{503}$
HALO-TMR

Figure S5. Microscopic analysis of SNAP-Qtip$^{L12A/D13A}$, SNAP-Qtip^{L12A}, SNAP-Qtip$^{L28A/L29A}$, SNAP-Qtip^{L29A}, Related to Figures 4 and 5.

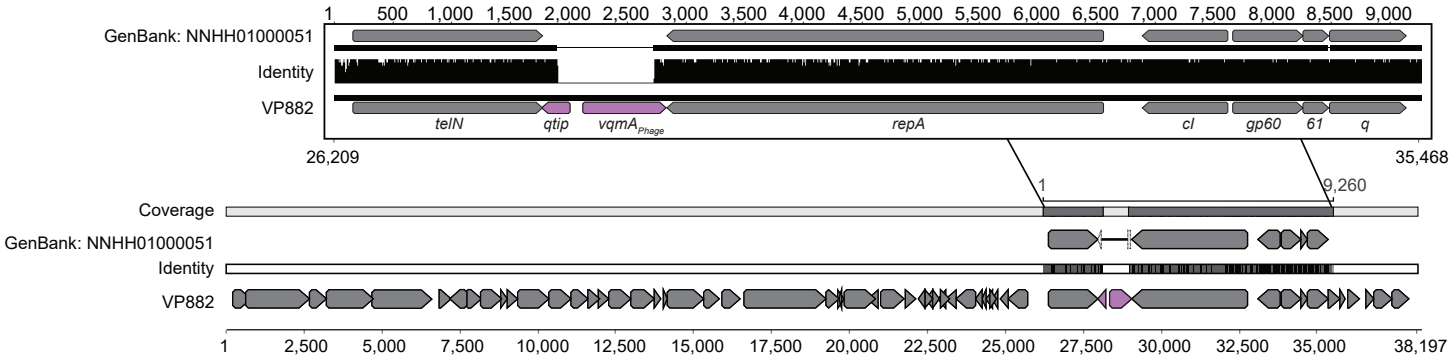
Composite images from individual cell analyses of *E. coli* producing cI$_{VP882}$-HALO and either SNAP-Qtip$^{L12A/D13A}$, SNAP-Qtip^{L12A} (A), or SNAP-Qtip$^{L28A/L29A}$, SNAP-Qtip^{L29A} (B). Samples labeled with SNAP-JF$_{503}$ and HALO-TMR, and displayed as in Figure 5B. Scale bars, as in Figure 1D.

FIGURE S6

A



B



C

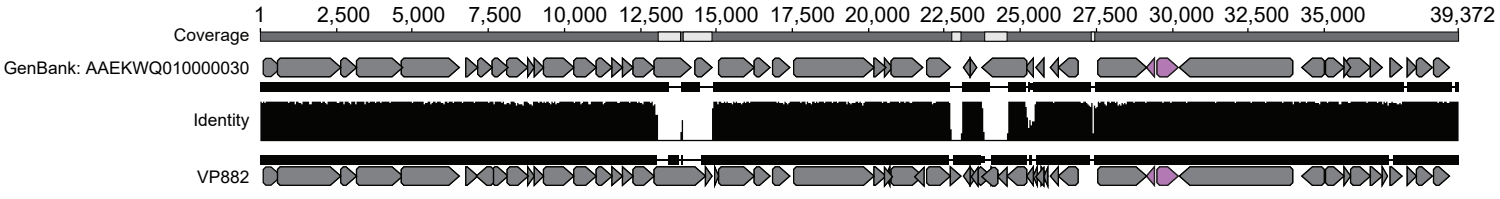


Figure S6. Ant does not induce P*q-lux* expression, and phage VP882-like elements are present in *Vibrio* and *Salmonella* isolates, Related to Figure 6.

(A) Light production from the P*q-lux* reporter in *E. coli* producing cI_{VP882} (no plasmid) or cI_{VP882} and a plasmid encoding Qtip or Ant. RLU as in Figure 1. Data represented as mean ± SD with n = 3 biological replicates. (B) Alignment of GenBank: NNHH01000051 across the VP882 phage genome. Arrows indicate ORFs, purple indicates *qtip* and $vqmA_{Phage}$. Top inset, magnified region of homology across the region containing *qtip* and $vqmA_{Phage}$. Identity plotted between the two alignments with a sliding window size of 1. The gap in the inset indicates the 819 bp deletion in the NNHH01000051 contig. Numbering below and above the alignment indicate the genomic coordinates in phage VP882 and the consensus sequence in the contig, respectively. (C) Alignment of the *Salmonella*-derived element (GenBank: AAEKWQ010000030) across the phage VP882 phage genome. Numbering above the alignment indicates coordinates in the consensus sequence. Identity, gaps, and color scheme as in (B).

Table S1. Bacterial strains used in this study, Related to STAR Methods.

Strain	Genotype	Reference
V. parahaemolyticus O3:K6	Wild-type; phage VP882 lysogen	BCRC 80155
E. coli T7Express lysY/I^q	*E. coli str. B, MiniF lysY lacIq(CamR) / fhuA2 lacZ::T7 gene1 [lon] ompT gal sulA11 R(mcr-73::miniTn10-TetS)2 [dcm] R(zgb- 210::Tn10-TetS) endA1 Δ(mcrC-mrr) 114::IS10*	NEB
E. coli TOP10	*F– mcrA Δ(mrr-hsdRMS-mcrBC) φ80lacZΔM15 ΔlacX74 recA1 araD139 Δ(ara-leu)7697 galU galK λ– rpsL(Str^R) endA1 nupG*	Invitrogen
E. coli cI857	*E. coli;* λ *cI857* lysogen	(Sussman and Jacob, 1962); gift of Tom Silhavy