Legends to Supplemental Figures

S1. Binding curves for the interactions of DNA targets with the *E. coli* TcpP-HSV$^+$ (TG387) membrane preparation. The % shifted values for each DNA target were plotted as a function of membrane protein concentration used in mobility shift experiments as presented in Fig. 3. The binding curves for the parental promoter target and derivatives carrying transversions at positions –56 through –48 (Panel A) and positions –47 through –38 (Panel B), as indicated on the right side of the figure, with a vertical arrow indicating the position of the pentameric repeat motif present in the region of the *toxT* promoter analyzed. The intercept of each binding curve with the dashed horizontal line drawn at 50% shifted indicates the concentration of membrane protein required for a 50% shift of each DNA target, as presented in Table 2.

S2. Binding curves for the interactions of DNA targets with the *V. cholerae* ToxR-HA$^+$ (TG129) membrane preparation. The % shifted values for each DNA target were plotted as a function of the membrane protein concentration used in mobility shift experiments as presented in S3. The binding curves for the parental promoter target and derivatives carrying transversions at positions –56 through –48 (Panel A) and positions –47 through –38 (Panel B), as indicated on the right side of the figure, with a vertical arrow indicating the position of the pentameric repeat motif present in the region of the *toxT* promoter analyzed. The intercept of each binding curve with the dashed horizontal line drawn at 50% shifted indicates the concentration of membrane protein required for a 50% shift of each DNA target, as presented in Table 3.

S3. Electrophoretic mobility shift assay for the binding of DNA targets to proteins present in *V. cholerae* ToxR$^-$ (TG128) and ToxR$^+$ (TG129) membrane preparations. The composite image was generated from a single piece of autoradiography film displaying migration patterns from five different gel runs (Panels A to E) as described in the legend to Fig. 3. For Panels A to E, the parental promoter (lanes 1, 2 and 3) or single transversion derivatives at positions –56 to –38 (lanes 4 to 22, respectively), are indicated at the top of the figure. The horizontal arrows above each panel indicate the positions of the pentameric repeat motifs within the TcpP-footprinted region of the *toxT* promoter. For lanes 3 to 22, the DNA-binding solutions containing end-labeled DNA targets were mixed with membrane buffer only (Panel A) or *V. cholerae* ToxR$^+$ membrane preparation at either 0.21 mg/ml (Panel B), 0.43 mg/ml (Panel C), 0.86 mg/ml (Panel D) or 1.7 mg/ml (Panel E), as indicated on the right side of the figure. DNA-membrane solutions were treated as described in the legend to Fig. 3. The positions of free and shifted end-labeled DNA target migration through the gel are indicated in the column between lanes 2 and 3. The % shifted values given below the free target signal for each sample indicate the percentage of shifted signal, relative to the sum of free and shifted signals, as quantified by densitometry. Control samples containing the parental target mixed with either membrane buffer only (lane 1) or negative control *V. cholerae* ToxR$^-$ membrane preparation (lane 2), present at the same protein concentration as the experimental membrane preparation used for lanes 3-22, as indicated on the right side of each panel, were included in each gel run where appropriate.

Figure S1

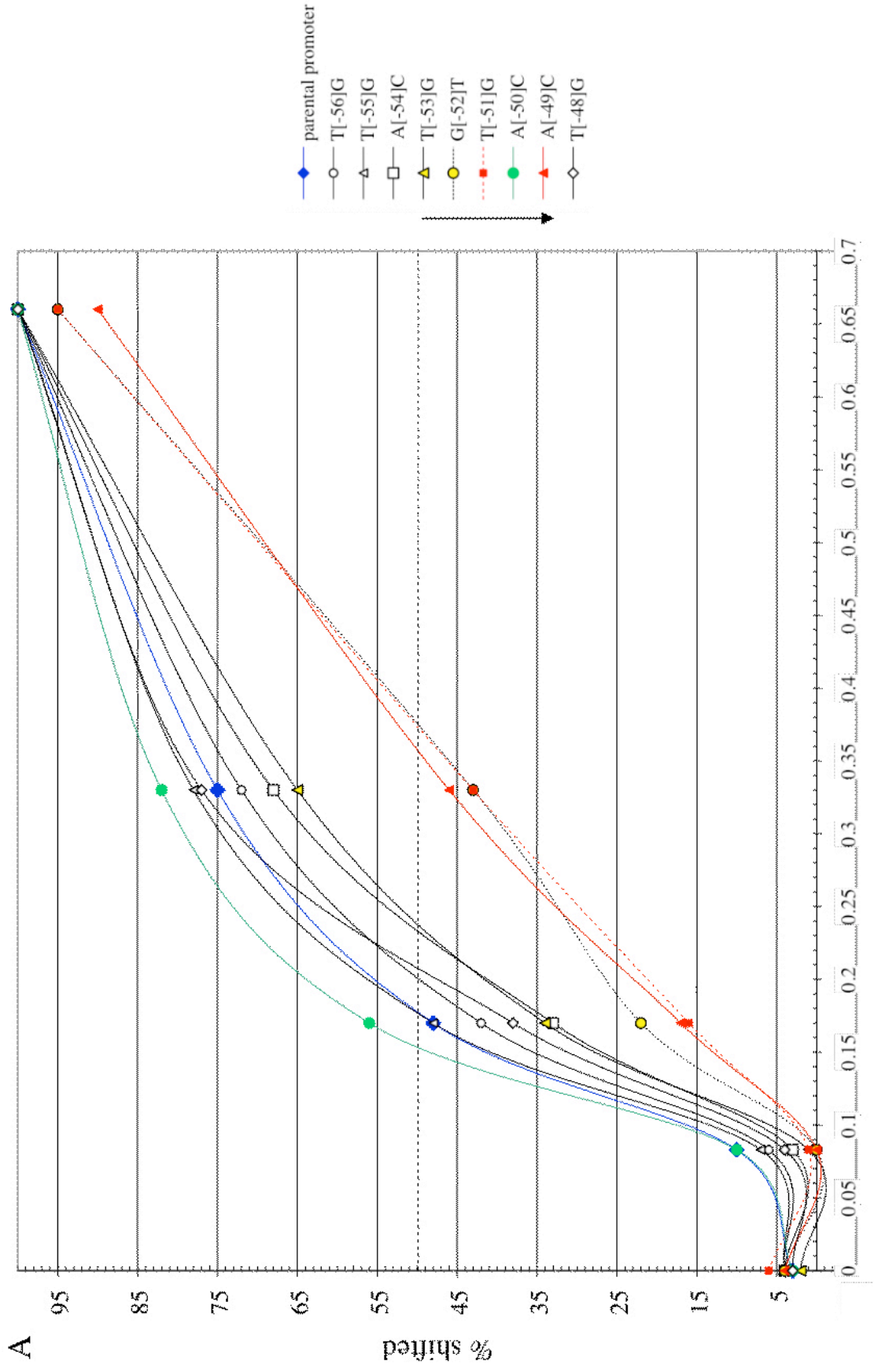

A
% shifted
95
85
75
65
55
45
35
25
15
5
0
0.05
0.1
0.15
0.2
0.25
0.3
0.35
0.4
0.45
0.5
0.55
0.6
0.65
0.7
mg TG387 (TcpP+) membrane protein/ml
parental promoter
T[-56]G
T[-55]G
A[-54]C
T[-53]G
G[-52]T
T[-51]G
A[-50]C
A[-49]C
T[-48]G

Figure S1 cont.

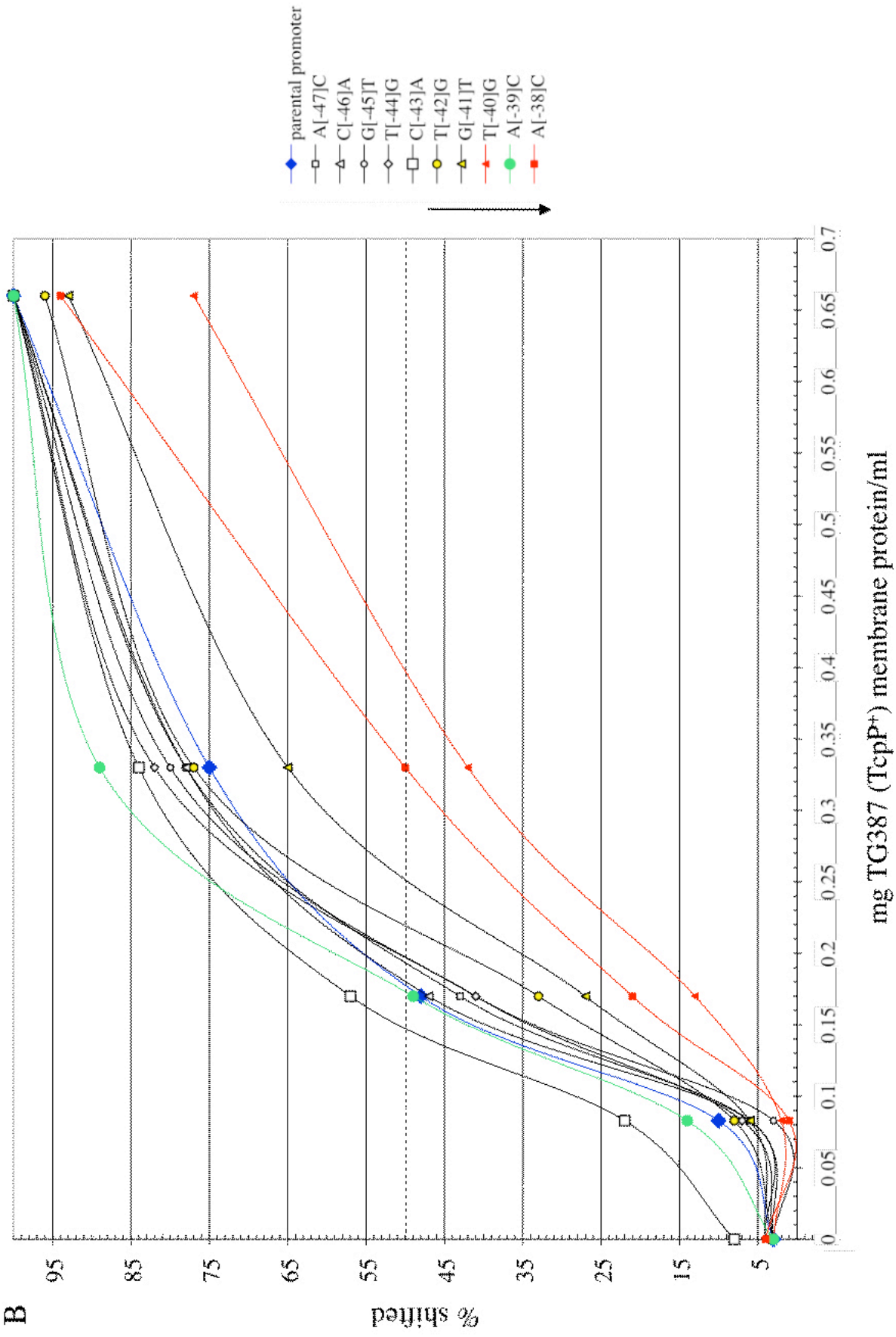


Figure S2

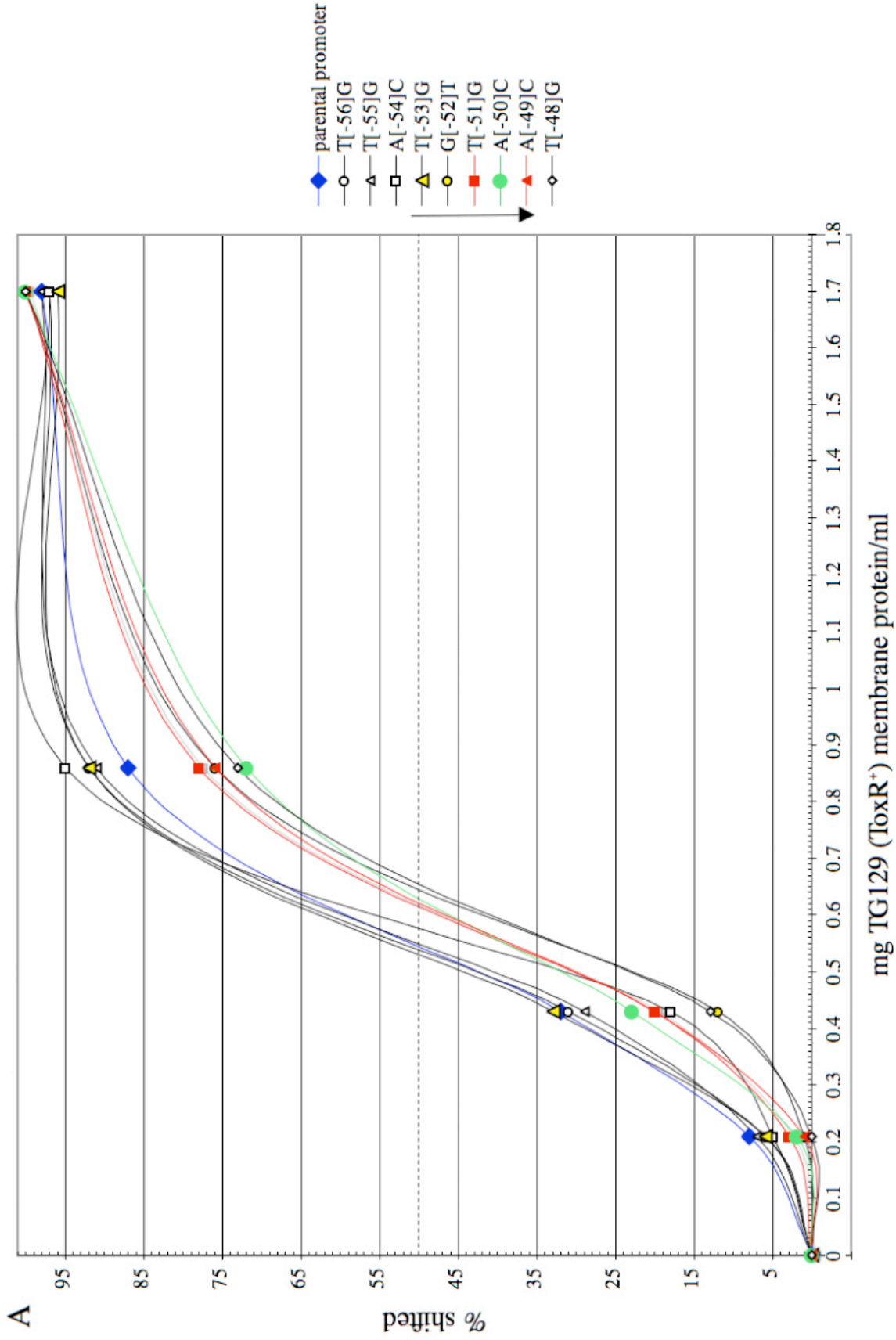

A
% shifted
95
85
75
65
55
45
35
25
15
5
0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8
0.9
1
1.1
1.2
1.3
1.4
1.5
1.6
1.7
1.8
mg TG129 (ToxR+) membrane protein/ml
parental promoter
T[-56]G
T[-55]G
A[-54]C
T[-53]G
G[-52]T
T[-51]G
A[-50]C
A[-49]C
T[-48]G

Figure S2 cont.

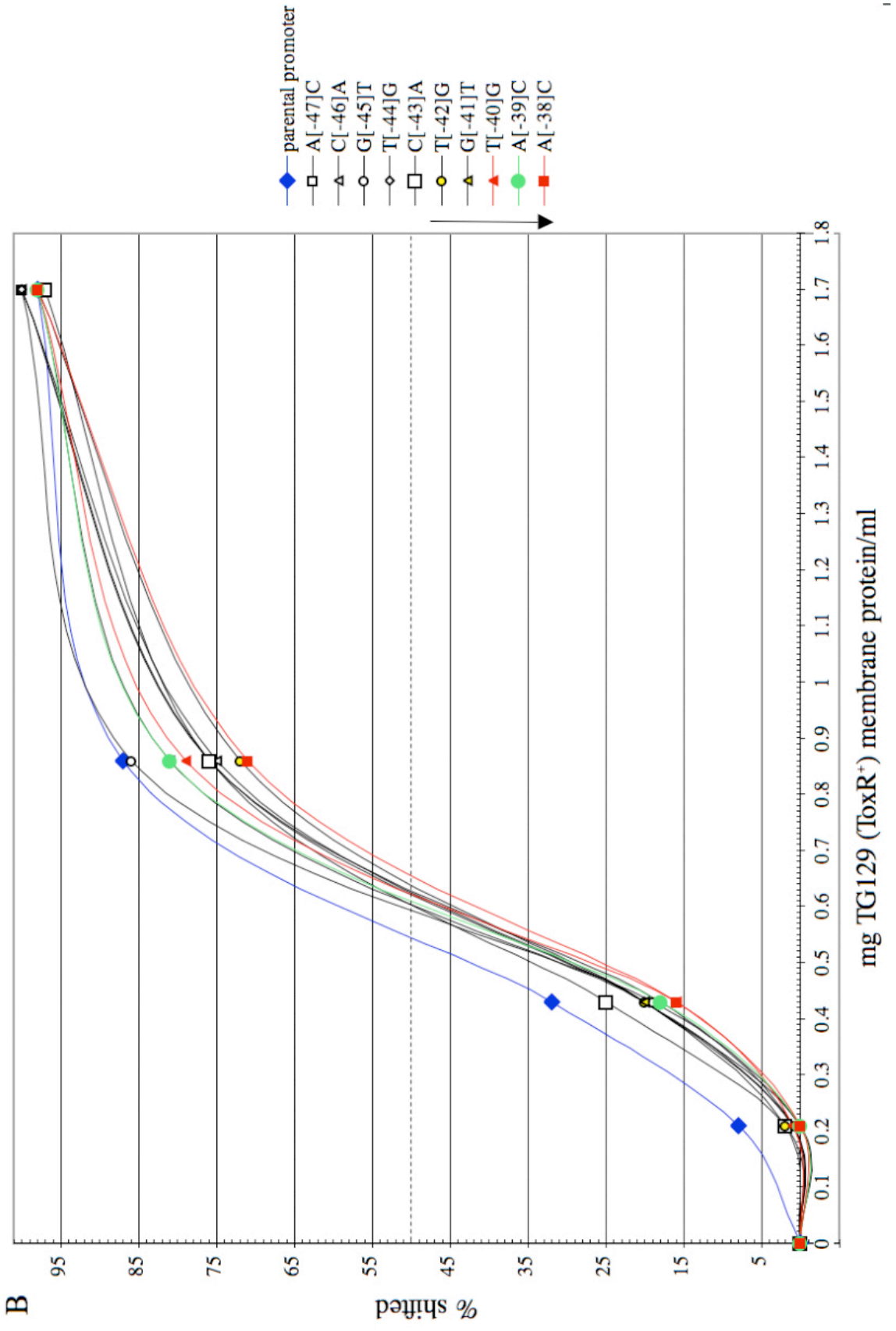
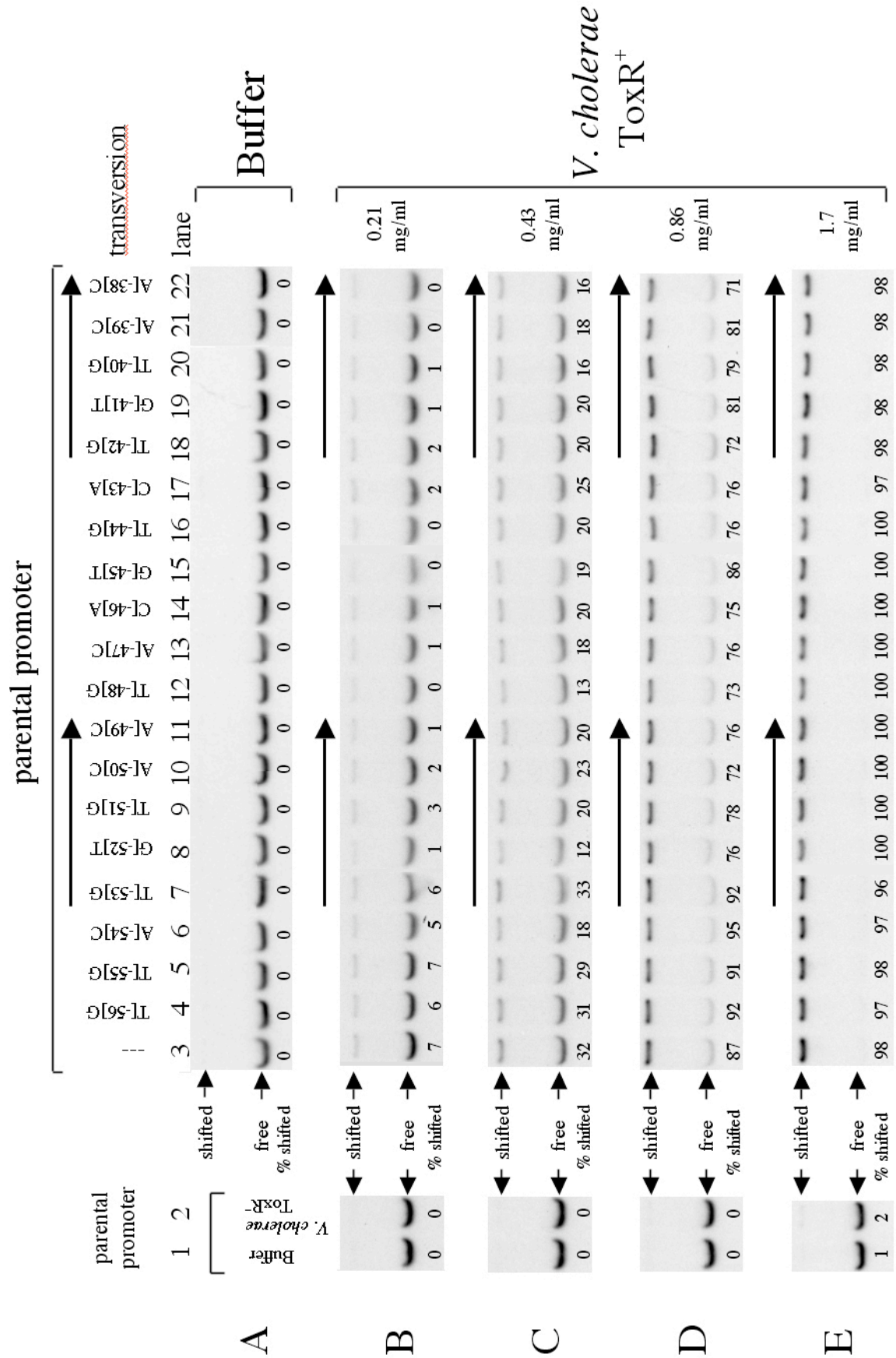


Figure S3


A
B
C
D
E
parental promoter
transversion
lane
Buffer
V. cholerae ToxR+
0.21 mg/ml
0.43 mg/ml
0.86 mg/ml
1.7 mg/ml
parental promoter
Buffer
V. cholerae ToxR-
shifted
free
% shifted
--- T[-56]G A[-55]G T[-54]C A[-53]G G[-52]T T[-51]G A[-50]C A[-49]C T[-48]G A[-47]C C[-46]A G[-45]T T[-44]G C[-43]A T[-42]G G[-41]T T[-40]G A[-39]C A[-38]C

1 Table S1: Oligonucleotides used in this study

primer name	purpose	sequence 5' to 3'
$toxT_{pro}$ -57 C-A TOP	mutagenesis	catgagttactttatgtttttattatgtaatacgtctgtaact
$toxT_{pro}$ -57 C-A BOTTOM	mutagenesis	agttacagacgtattacataataaaaacataaagtaactcatgttat
$toxT_{pro}$ -56 T-G TOP	mutagenesis	tactttatgtttcgagtatgtaatacgtctg
$toxT_{pro}$ -56 T-G BOTTOM	mutagenesis	cagacgtattacatactcgaaacataaagta
$toxT_{pro}$ -55 T-G TOP	mutagenesis	actttatgtttcgatgatgtaatacgtctgt
$toxT_{pro}$ -55 T-G BOTTOM	mutagenesis	acagacgtattacatcatcgaaacataaagt
$toxT_{pro}$ -54 A-C TOP	mutagenesis	ctttatgtttcgattctgtaatacgtctgta
$toxT_{pro}$ -54 A-C BOTTOM	mutagenesis	tacagacgtattacagaatcgaaacataaag
$toxT_{pro}$ -53 T-G TOP	mutagenesis	tttatgtttcgattaggtaatacgtctgtaa
$toxT_{pro}$ -53 T-G BOTTOM	mutagenesis	ttacagacgtattacctaatcgaaacataaa
$toxT_{pro}$ -52 G-T TOP	mutagenesis	ttatgtttcgattatttaatacgtctgtaac
$toxT_{pro}$ -52 G-T BOTTOM	mutagenesis	gttacagacgtattaaataatcgaaacataa
$toxT_{pro}$ -51 T-G TOP	mutagenesis	tatgtttcgattatggaatacgtctgtaact
$toxT_{pro}$ -51 T-G BOTTOM	mutagenesis	agttacagacgtattccataatcgaaacata
$toxT_{pro}$ -50 A-C TOP	mutagenesis	atgtttcgattatgtcatacgtctgtaactt
$toxT_{pro}$ -50 A-C BOTTOM	mutagenesis	aagttacagacgtatgacataatcgaaacat
$toxT_{pro}$ -49 A-C TOP	mutagenesis	tgtttcgattatgtactacgtctgtaacttg
$toxT_{pro}$ -49 A-C BOTTOM	mutagenesis	caagttacagacgtagtacataatcgaaaca
$toxT_{pro}$ -48 T-G TOP	mutagenesis	tgtttcgattatgtaagacgtctgtaacttgttcttatgtct
$toxT_{pro}$ -48 T-G BOTTOM	mutagenesis	agacataagaacaagttacagacgtcttacataatcgaaaca
$toxT_{pro}$ -47 A-C TOP	mutagenesis	tgtttcgattatgtaatccgtctgtaacttgttcttatgtct
$toxT_{pro}$ -47 A-C BOTTOM	mutagenesis	agacataagaacaagttacagacggattacataatcgaaaca
$toxT_{pro}$ -46 C-A TOP	mutagenesis	tgtttcgattatgtaataagtctgtaacttgttcttatgtct
$toxT_{pro}$ -46 C-A BOTTOM	mutagenesis	agacataagaacaagttacagacttattacataatcgaaaca
$toxT_{pro}$ -45 G-T TOP	mutagenesis	tcgattatgtaatacttctgtaacttgttct
$toxT_{pro}$ -45 G-T BOTTOM	mutagenesis	agaacaagttacagaagtattacataatcga
$toxT_{pro}$ -44 T-G TOP	mutagenesis	cgattatgtaatacggctgtaacttgttctt
$toxT_{pro}$ -44 T-G BOTTOM	mutagenesis	aagaacaagttacagccgtattacataatcg
$toxT_{pro}$ -43 C-A TOP	mutagenesis	gattatgtaatacgtatgtaacttgttctta
$toxT_{pro}$ -43 C-A BOTTOM	mutagenesis	taagaacaagttacatacgtattacataatc
$toxT_{pro}$ -42 T-G TOP	mutagenesis	attatgtaatacgtcggtaacttgttcttat
$toxT_{pro}$ -42 T-G BOTTOM	mutagenesis	ataagaacaagttaccgacgtattacataat
$toxT_{pro}$ -41 G-T TOP	mutagenesis	ttatgtaatacgtctttaacttgttcttatg
$toxT_{pro}$ -41 G-T BOTTOM	mutagenesis	cataagaacaagttaaagacgtattacataa
$toxT_{pro}$ -40 T-G TOP	mutagenesis	tatgtaatacgtctggaacttgttcttatgt
$toxT_{pro}$ -40 T-G BOTTOM	mutagenesis	acataagaacaagttccagacgtattacata
$toxT_{pro}$ -39 A-C TOP	mutagenesis	atgtaatacgtctgtcacttgttcttatgtc
$toxT_{pro}$ -39 A-C BOTTOM	mutagenesis	gacataagaacaagtgacagacgtattacat

toxT$_{pro}$ -38 A-C TOP	mutagenesis	tgtaatacgtctgtaccttgttcttatgtct
toxT$_{pro}$ -38 A-C BOTTOM	mutagenesis	agacataagaacaaggtacagacgtattaca
toxT$_{pro}$ -51 T-G, -40 T-G TOP	mutagenesis	tatgtttcgattatggaatacgtctggaacttgttcttatgt
toxT$_{pro}$ -51 T-G, -40 T-G BOTTOM	mutagenesis	acataagaacaagttccagacgtattccataatcgaaacata
toxT$_{pro}$ -49 A-C, -38 A-C TOP	mutagenesis	tgtttcgattatgtactacgtctgtaccttgttcttatgtct
toxT$_{pro}$ -49 A-C, -38 A-C BOTTOM	mutagenesis	agacataagaacaaggtacagacgtagtacataatcgaaaca
toxT$_{pro}$ -51 T-G, -49 A-C, -40 T-G, -38 A-C TOP	mutagenesis	tatgtttcgattatggactacgtctggaccttgttcttatgtct
toxT$_{pro}$ -51 T-G, -49 A-C, -40 T-G, -38 A-C BOTTOM	mutagenesis	agacataagaacaaggtccagacgtagtccataatcgaaacata
toxT$_{pro}$ -50 A-C, -39 A-C TOP	mutagenesis	tgtttcgattatgtcatacgtctgtcacttgttcttatgtct
toxT$_{pro}$ -50 A-C, -39 A-C BOTTOM	mutagenesis	agacataagaacaagtgacagacgtatgacataatcgaaaca
5’ *Eco*RI *toxT* #12	cloning primer	cccggaattcgaaaatggtcgatatgat
3’ *Eco*RI *toxT* #15	cloning primer	ccggaattctactttcgagaagaaccc
toxT$_{pro}$ -172 *Bam*HI	cloning primer	gcgcggatccgtatagcaaagcatattcagagaac
toxT$_{pro}$ +45 *Eco*RI	cloning primer	gcgcgaattcaaataaacgcagagagccatcc
*Xho*I to *Not*I	pTL61T *Xho*I site to *Not*I site conversion	tcgacgcggccgcg
wt-TcpP + basal TOP	construction of parental *toxT* promoter	cgattatgtaatacgtctgtaacttgttcttatgtctgttatgtagttttatattaactt
wt-TcpP + basal BOTTOM	construction of parental *toxT* promoter	aagttaatataaaactacataacagacataagaacaagttacagacgtattacataatcg
ToxR binding site TOP	construction of parental *toxT* promoter	tggctgttatcttatctcaaaaaacataaaataacatgagttactttatgttt
ToxR binding site BOTTOM	construction of parental *toxT* promoter	aaacataaagtaactcatgttatttttatgttttttgagataagataacagcca
5’*toxR-HA*	epitope tagging of *toxR*	gggggatcctcaaaagagatatcgatgag
3’ *toxR-HA*	epitope tagging of *toxR*	gggggctcgagctcacacactttgatggc

2