The 5’ UTR of the type I toxin ZorO can both inhibit and enhance translation

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Supplemental Table S1. Strains and Plasmids used in this study.

Name	Relevant genotype or description	Source
Escherichia coli EDL933	*E. coli* O157:H7, EHEC	D. Friedman
DJ480	MG1655 Δ*lacX74*	D. Jin, (55)
UTK007	DJ480 PCP18-*araE*	(18)
UTK011	UTK007 Δ*rnc::kan*	(18)
Plasmids		
pFA6a-GFP(S65T)-kanMX6	Amp^R; Km^R	(23)
pEF21	Cm^R; P_{BAD} promoter	(9)
pEF21-*zorO* full-length	Cm^R	(18)
pEF21-*zorO* Δ28	Cm^R	This study
pAZ3	Cm^R; P_{BAD} promoter	(8)
pAZ3-*zorO* full-length	Cm^R	This study
pAZ3-*zorO* Δ28	Cm^R	This study
pAZ3-*zorO* Δ34	Cm^R	This study
pAZ3-*zorO* Δ50	Cm^R	This study
pAZ3-*zorO* Δ82	Cm^R	This study
pAZ3-*zorO* Δ82 RBS	Cm^R	This study
pAZ3-*zorO* UTR-*gfp*	Cm^R	This study
pAZ3-Δ28 UTR-*gfp*	Cm^R	This study
pBR-pLac	Amp^R; P_{LlacO} promoter	(55)
pBR-pLac-*orzO*	Amp^R	(18)
pGEM®-3Zf(+)	Amp^R; T7 promoter	Promega
pGEM-T7-*zorO* full-length	Amp^R	This study
pGEM-T7-*zorO* Δ50	Amp^R	This study
pGEM-T7-*zorO* Δ82	Amp^R	This study
pGEM-T7-*zorO* Δ82 RBS	Amp^R	This study

Supplementary Table S2. Oligonucleotides used in this study.

Name	Sequence[a]	Use
EF524	GTTGGTACGAAACGTTGCTCTCCG	Northern analysis of *zorO*, *zorO* UTR-*gfp* and their derivatives
EF1432	CGGCGCTACGGCGTTTCACTTCTG	Northern analysis of 5s
EF1065	CAGTGAGTGAGATTTATAATCGAATTCGTTGGGAG	pAZ3-*zorO* full-length PCR; pAZ3-*zorO* UTR-*gfp* PCR; pGEM-T7-*zorO* full-length PCR
EF1068	ATACTCAAGCTTATTAAAGTCGCAGCACATGCAAC	pAZ3-*zorO* full-length PCR; pAZ3-*zorO* Δ28 PCR; pAZ3-*zorO* Δ50 PCR; pAZ3-*zorO* Δ82 PCR; pEF21-*zorO* Δ28 PCR
EF1141	GGTTGTGCCGGATCGGAATTCTTTTAAGTCCTGGCTGC	pAZ3-*zorO* Δ28 PCR; pAZ3-Δ28 UTR-*gfp* PCR
EF1127	GACCAAGAATTCGTCCTGGCTGCCGGACGGGTGGTGCCGC	pAZ3-*zorO* Δ34 PCR
EF1066	GATAATTAAGCTTGCAGCACATGCAACTTGAAG	pAZ3-*zorO* Δ34 PCR
EF1170	CAATTTTAAGTCCTGGCTGGAATTCGGGTGGTGCCGCAGGC	pAZ3-*zorO* Δ50 PCR; pGEM-T7-*zorO* Δ50 PCR
EF392	GCCCTGGAATTCAGAGCAACGTTTCGTACCAAC	pAZ3-*zorO* Δ82 PCR; pGEM-T7-*zorO* Δ82 PCR
EF1290	GTGTAAGGGTAAGGTGCTGGTGTTGCG**TTGGTA**AATAAGGAGAGCGGATGGACACGCTGAC	pAZ3-*zorO* Δ82 RBS PCR; pGEM-T7-*zorO* Δ82 RBS PCR
EF1291	GTCAGCGTGTCCATCCGCTCTCCTTATT**TACCAA**CGCAACACCAGCACCTTACCCTTACAC	pAZ3-*zorO* Δ82 RBS PCR; pGEM-T7-*zorO* Δ82 RBS PCR
EF1181	GAAAAGTTCTTCTCCTTTCATCCGCTCTCCTTATTAAGG	pAZ3-*zorO* UTR-*gfp* PCR
EF1182	CCTTAATAAGGAGAGCGGATGAAAGGAGAAGAACTTTTC	pAZ3-*zorO* UTR-*gfp* PCR
EF1183	GAAATTCGCTTATTTAGAAGCTTCGCGCCCTATTTGTATAG	pAZ3-*zorO* UTR-*gfp* PCR
EF1328	GGTTGTGCCGGATCGCTGCAGTTTTAAGTCCTGGCTGC	pEF21-*zorO* Δ28 PCR
EF1343	GAGTATAGCTCCCGGGAAGGGGGAAACGGTATTC	pGEM- T7 *zorO* full-length PCR; pGEM-T7-*zorO* Δ50 PCR; pGEM-T7-*zorO* Δ82 PCR; T7-*zorO* Δ28 PCR
EF1408	GAGTCTGCAGAAGCTTTAATACGACTCACTATAGGGCGAATTCTTTTAAGTCCTGGCTGCCGGACGGGTG	T7-*zorO* Δ28 PCR

[a] 5' - 3', restriction sites underlined, nucleotides altered via site directed mutagenesis in red.

Supplemental Figure S1. No difference in fluorescence intensity between the uninduced controls of pAZ3- *zorO* UTR-*gfp* and pAZ3-Δ28 UTR-*gfp*. Shown are flow cytometry analyses of the fluorescence intensity of GFP from cells harboring pAZ3- *zorO* UTR-*gfp* or pAZ3-Δ28 UTR-*gfp* without arabinose induction. Lines indicate time post induction. Shown is a representative of three independent experiments.

Supplemental Figure S2. *In vitro* structure probing of 3'-end-labeled *zorO* and its derivatives. 3′-end-labeled *zorO* full-length, *zorO* Δ28, *zorO* Δ50, or *zorO* Δ82 RNA (1.67 μM) was subjected to RNase T1 and lead(II) acetate cleavage as outlined in Figure 3A. The position of selected cleaved G residues is given at the left of the T1 ladder. Yellow lines indicate the corresponding RBS of *zorO* full-length, *zorO* Δ28, *zorO* Δ50, and *zorO* Δ82. For clarity, all positions are given as for *zorO* full-length mRNA from the 5' end of the transcript even though nucleotide numbers might be different in mutant RNAs.

Supplemental Figure S3. Structural changes in the full-length versus *zorO* Δ28. **(A)** Schematic of structural changes upon processing of *zorO* mRNA. Removal of the first 28 nt (indicated by black bracket) in the *zorO* full-length mRNA during processing leads to opening of the EAP region. For simplicity, only part of the *zorO* 5' UTR putative structure is shown. Due to some variable cleavage by RNase T1, there may be some weak structural elements that are not accounted for in this schematic. **(B)** Variable cleavage by RNase TI. Structure probing was done as in Figure 3B. Sample positions that showed experimental variation between structure probing experiments are as follows: yellow circles indicate G107 (*zorO* Δ28 is more sensitive in this example compared to itself in Figure 3B; the full-length is less sensitive compared to itself in as seen in Figure 3B), green circles indicate G95 (full-length is less sensitive in this example compared to itself in Figure 3B, while *zorO* Δ28 is equivalent); the same positions are indicated in Figure 3B for comparison. Position G75 (blue circles) is indicative of a residue whose cleavage was consistent across experiments. Note that the degree of sensitivity to cleavage may be variable between experiments; however, in all cases the residues indicated above were digested in *zorO* Δ28.

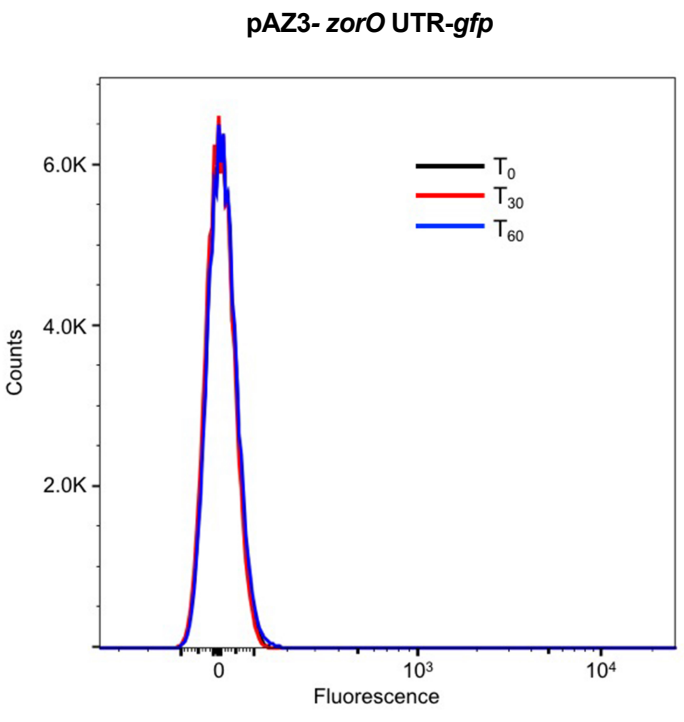
Supplemental Figure S4. Schematic diagram of the *zorO* Δ82 RBS mutant. **(A)** To generate the *zorO* Δ82 RBS mutant, the region (indicated by the black bar) that pairs with the RBS (indicated by yellow box) in the *zorO* Δ82 were mutated to release the RBS from the stem structure. Mutated nucleotides are shown in red. **(B)** Putative structure of the *zorO* Δ82 RBS mutant generated by Mfold (31). The RBS is indicated by yellow box and the region originally base pairing with the RBS is indicated by black bar.

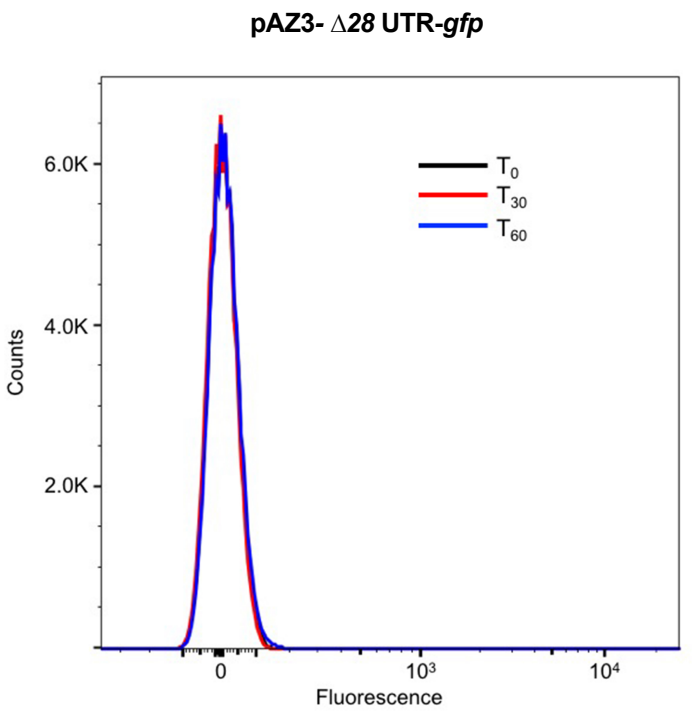
Supplemental Figure S5. OrzO sRNA rescues cells from *zorO* full-length or *zorO* Δ28 induced toxicity in *E. coli* strain UTK007 (wild type). **(A)** *E. coli* strain UTK011 (Δ*rnc*) carrying pBR-plac-*orzO* and pEF21-

zorO was induced as indicated with arabinose (0.002%) and/or IPTG (1mM). Shown are the mean values ± standard deviations for three independent cultures. **(B)** Rescue experiment was performed as in (A) in *E. coli* strain UTK007 (wild type). Shown are the mean values ± standard deviations for three independent cultures. **(C)** Rescue experiment was performed as in *E. coli* strain UTK007 (wild type) with *zorO* Δ28 expressed induced by 0.00002% arabinose and/or IPTG (1 mM). Shown are the mean values ± standard deviations for three independent cultures.

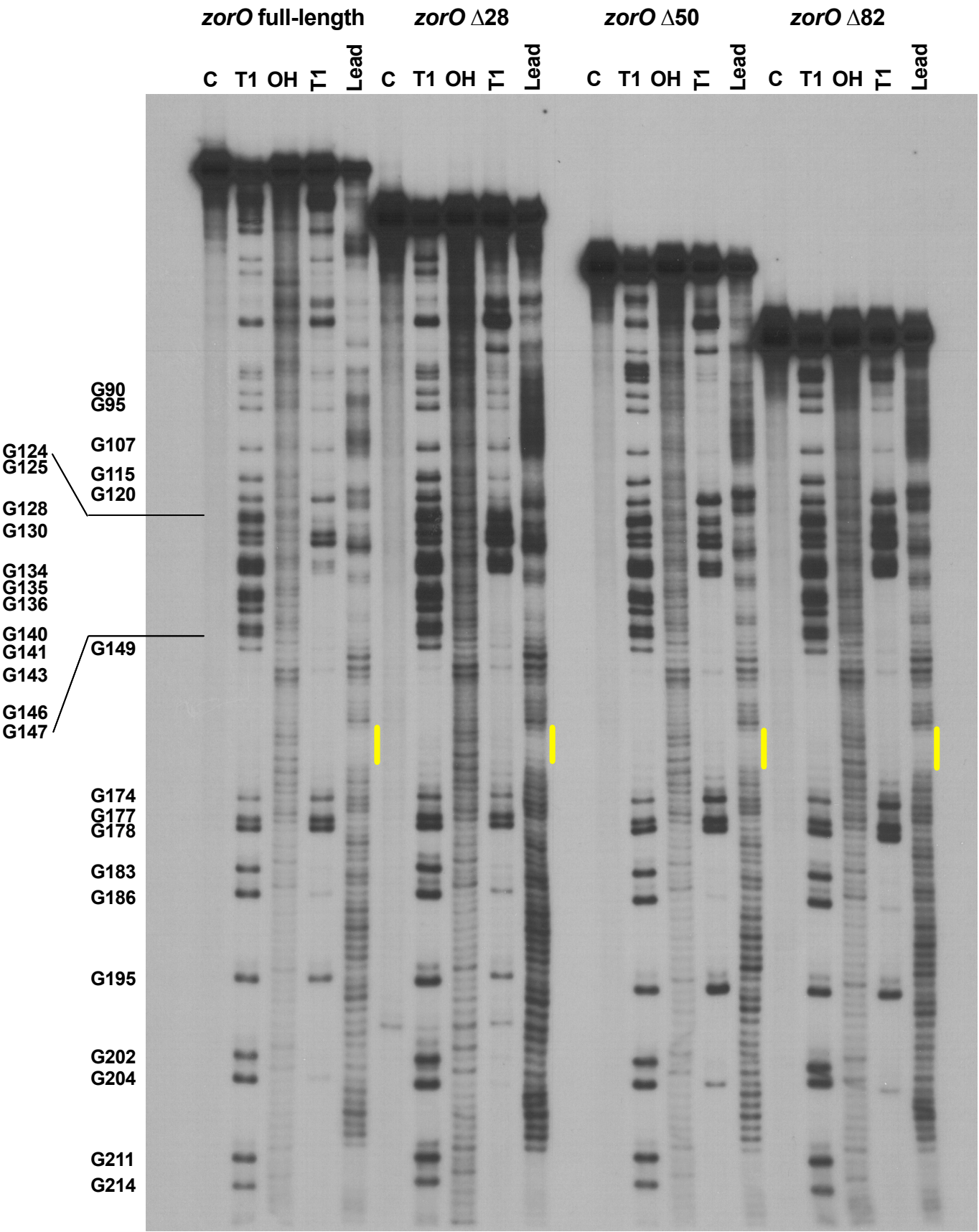
Supplemental Figure S6. The +35 to +50 region in *zorO* full-length and *zorO* Δ28. *In vitro* structure probing was conducted as in Figure 3A. Purple bars indicate the corresponding +35 to +50 region of *zorO* full-length and *zorO* Δ50.

Supplemental Figure S1



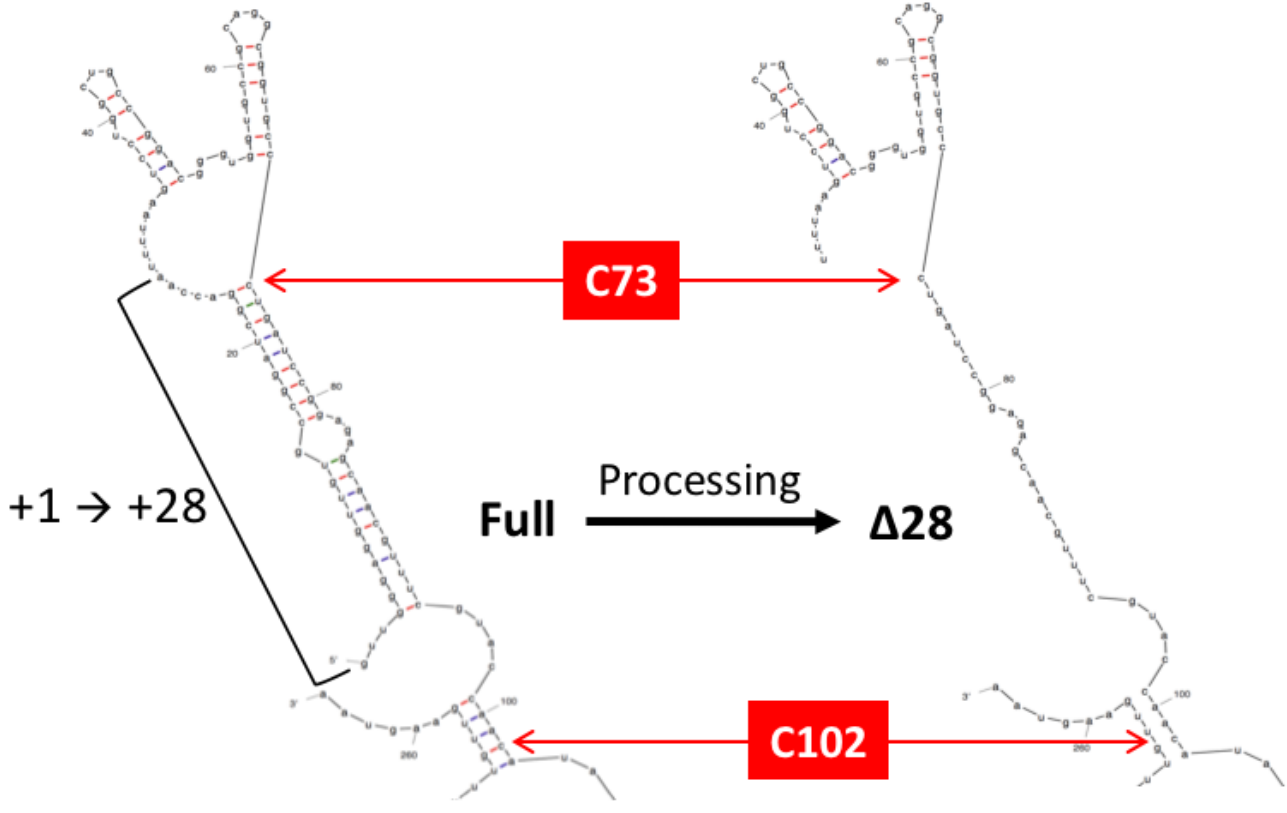


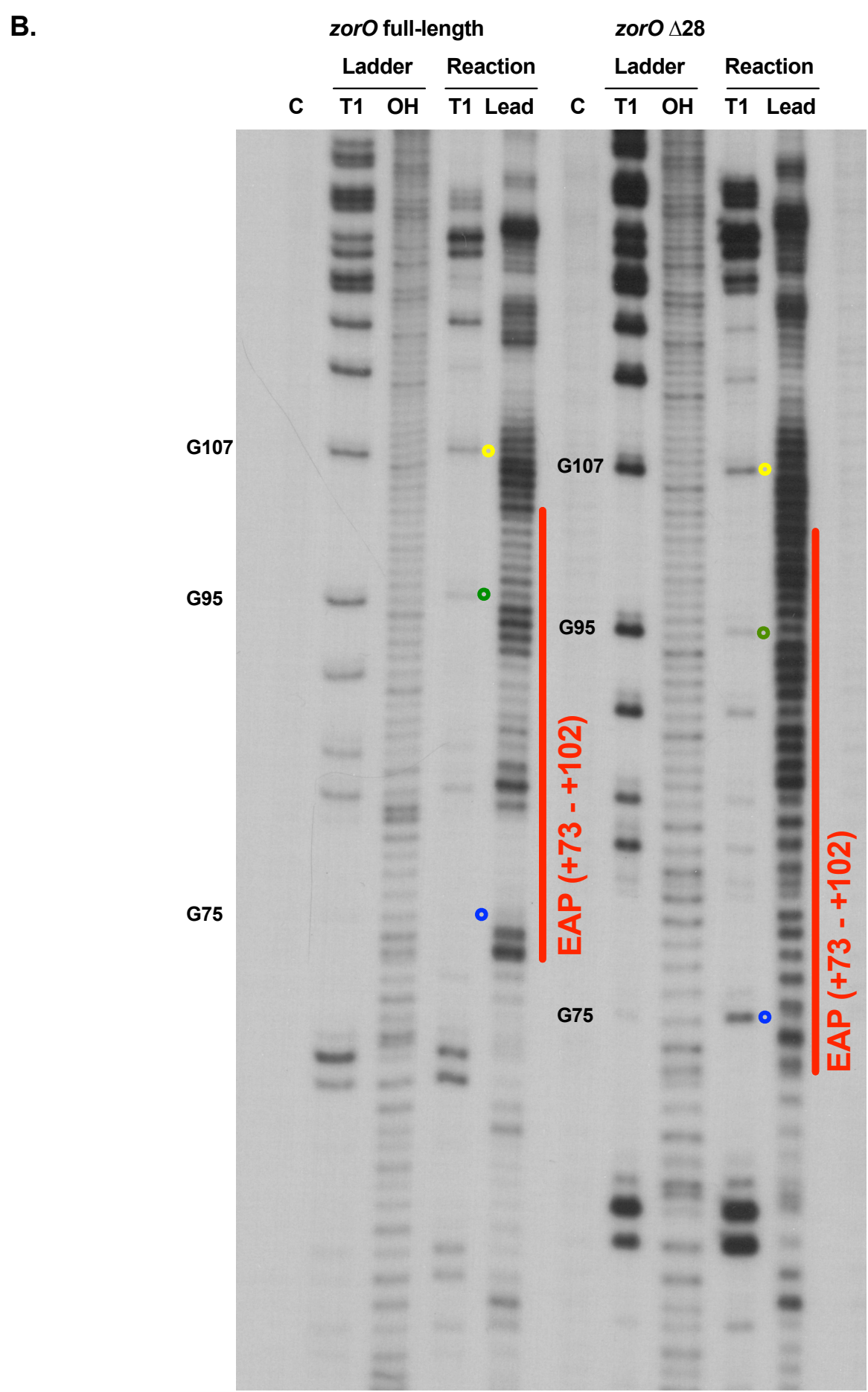
Supplemental Figure S2



Supplemental Figure S3A

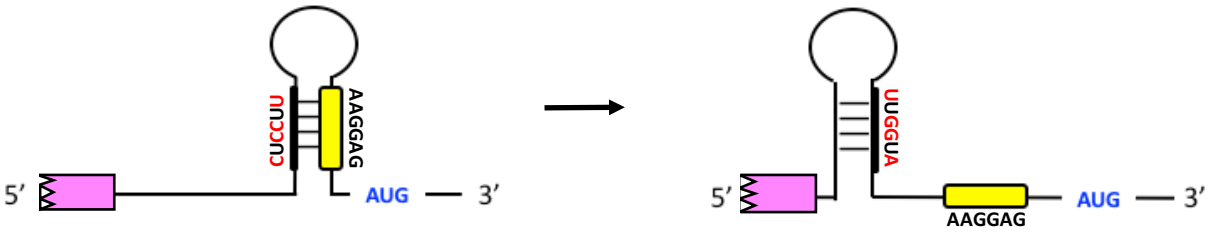
A.




B.
zorO full-length
Ladder
Reaction
C
T1
OH
T1
Lead
zorO Δ28
Ladder
Reaction
C
T1
OH
T1
Lead
G107
G95
G75
EAP (+73 - +102)
G107
G95
G75
EAP (+73 - +102)

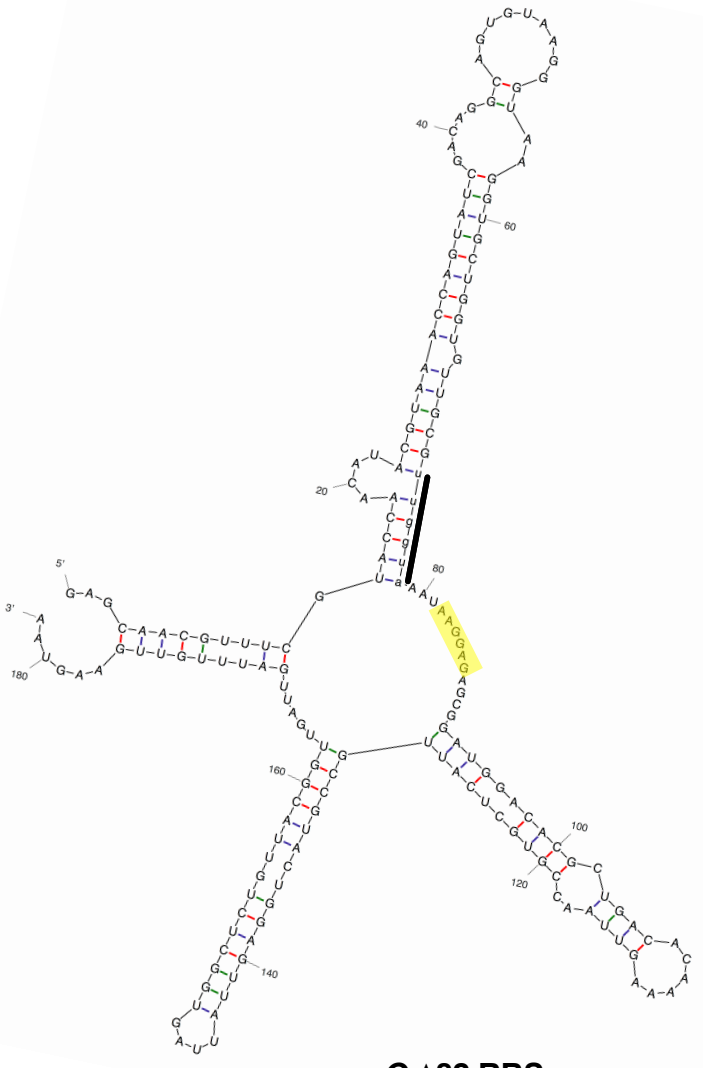
Supplemental Figure S4

A.



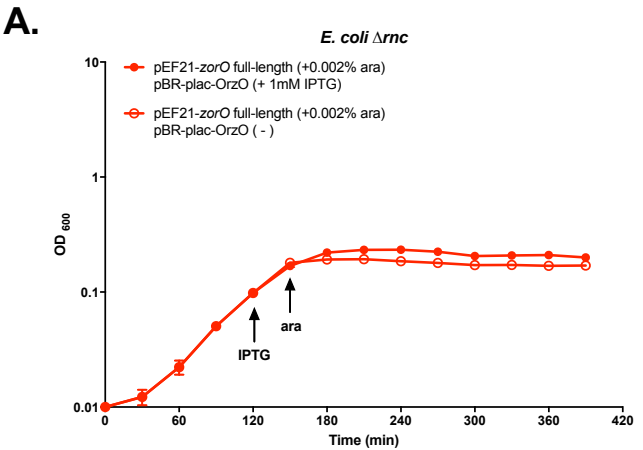
***zorO* Δ82** **zorO Δ82 RBS**

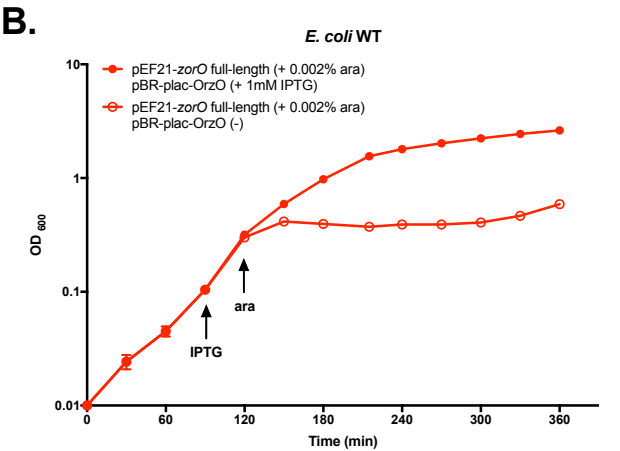
B.

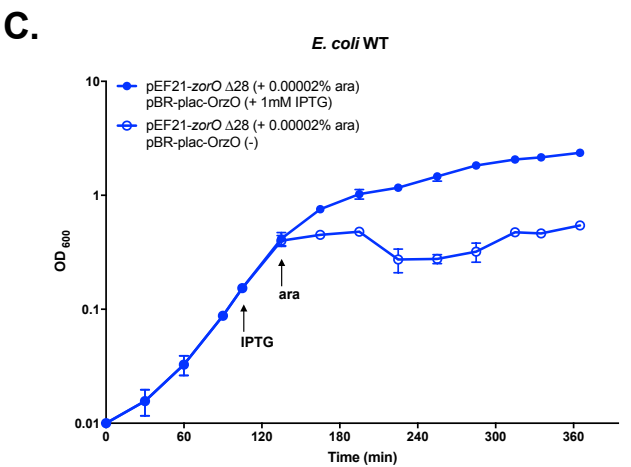


***zorO* Δ82 RBS**

Supplemental Figure S5







Supplemental Figure S6