Expanded View Figures

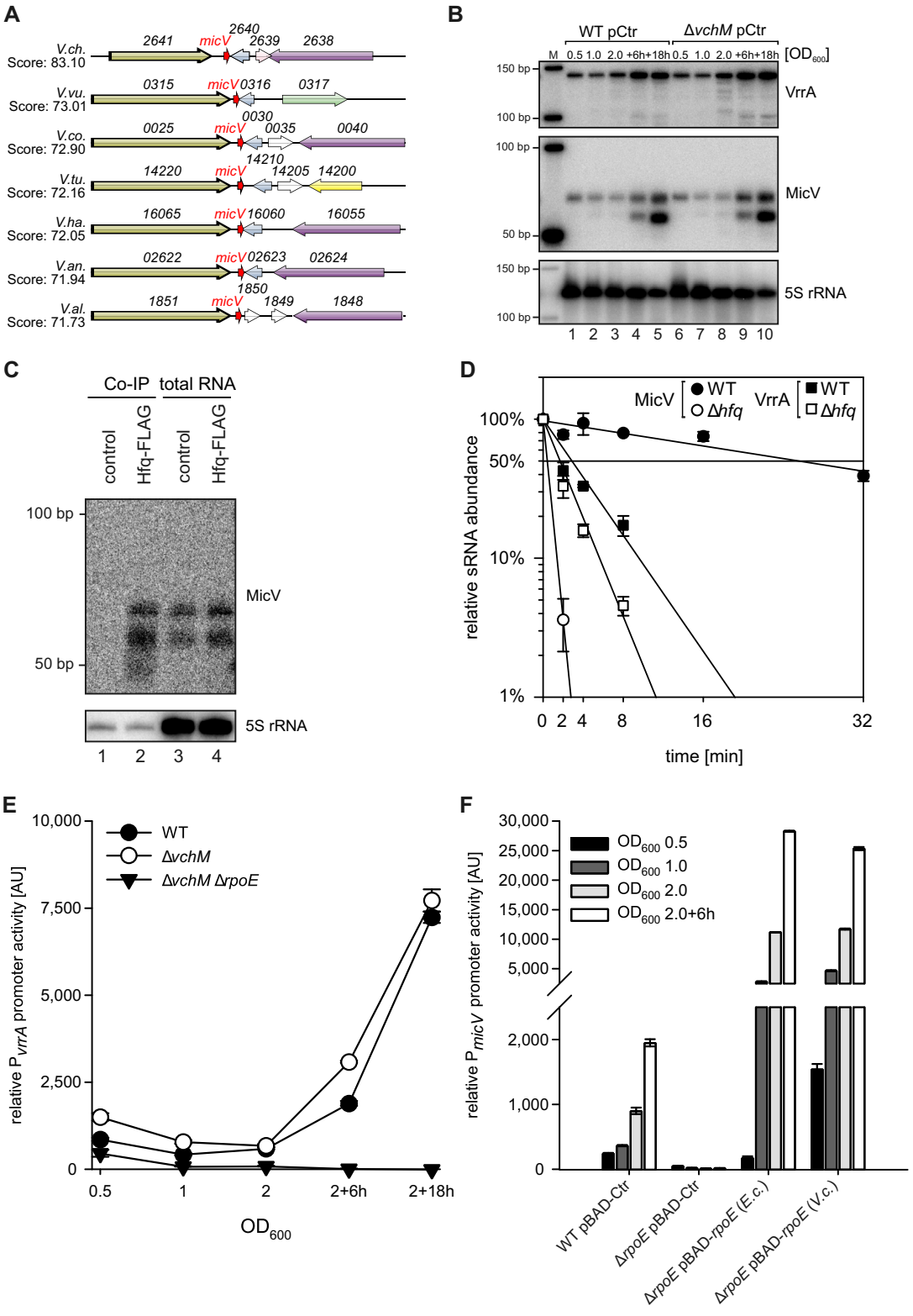

Figure EV1.

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Figure EV1. Genomic context and conserved transcriptional control of σ^E-dependent sRNAs in *V. cholerae* (related to Fig 1).

A Gene synteny analysis between the genomic loci encoding *micV* in various *Vibrio* strains. Homologous genes are indicated by the same colors.

B *Vibrio cholerae* wild-type and Δ*vchM* strains carrying empty vector control plasmids (pCtr) were grown in LB medium. At the indicated time points, RNA samples were collected and tested for *micV* and *vrrA* expression by Northern blot analysis. A size marker is provided on the left (M), and 5S rRNA was used as loading control.

C *Vibrio cholerae* wild-type (control) and *hfq::hfq-3xFLAG* (Hfq-FLAG) strains were grown to stationary phase (OD_{600} of 2), lysed, and subjected to immunoprecipitation using the anti-FLAG antibody. RNA samples of lysate (total RNA) and co-immunoprecipitated fractions were analyzed on Northern blots. 5S rRNA served as loading control.

D *Vibrio cholerae* wild-type and Δ*hfq* strains were cultivated in LB medium to an OD_{600} of 1.0. Cells were treated with rifampicin to terminate transcription. Total RNA samples were collected at the indicated time points, and MicV or VrrA transcript levels were monitored on Northern blots.

E *Vibrio cholerae* wild-type, Δ*vchM,* and Δ*vchM* Δ*rpoE* strains harboring P*vrrA::mKate2* plasmids were grown in M9 minimal medium. Samples were collected at various stages of growth and analyzed for fluorescence.

F *Escherichia coli* BW25113 wild-type and Δ*rpoE* strains carrying P*micV::gfp* plasmids and either empty vector control (pBAD-Ctr) or plasmids expressing *rpoE* of *E. coli* (pBAD-*rpoE* (*E.c*)) or of *V. cholerae* (pBAD-*rpoE* (*V.c*)) were grown in LB medium, supplemented with L-arabinose (0.2% final conc.). Samples were collected at various stages of growth and analyzed for fluorescence.

Data information: In (D–F), data are presented as mean ± SD, $n = 3$.

Source data are available online for this figure.

Figure EV2. VrrA harbors two conserved base-pairing regions to regulate mRNA targets (related to Fig 3).

A Alignment of the *vrrA* sequences of several *Vibrio* species. The boxes indicate the conserved seed regions R1 and R2. Mutations used in (B, D, E) are indicated.

B *Vibrio cholerae* Δ*vrrA* Δ*micV* strains carrying pMicV, pMicV M1, pVrrA, pVrrA M1, pVrrA M2, or an empty vector control (pCtr) were grown to OD_{600} of 1.0 in LB medium. RNA samples were collected and monitored for *micV* and *vrrA* expression by Northern blot analysis. 5S rRNA served as loading control.

C *Vibrio cholerae* Δ*rpoE*, Δ*vrrA* Δ*rpoE*, Δ*micV* Δ*rpoE*, or Δ*vrrA* Δ*micV* Δ*rpoE* strains carrying pBAD-*rpoE* plasmids or an empty vector control (pCtr) were grown to early stationary phase (OD_{600} of 1.5), and *rpoE* expression was induced by treatment with L-arabinose (0.2% final conc.). RNA samples were collected at the indicated time points and monitored for *micV* and *vrrA* expression by Northern blot analysis. 5S rRNA served as loading control.

D Predicted base-pairing of VrrA with the 5′UTR of *ompT*.

E *Vibrio cholerae* Δ*vrrA* Δ*micV* strains carrying *ompT::gfp* or *ompT* M1*::*gfp* fusions and an empty vector control (pCtr) or *vrrA* expression plasmids (pVrrA, pVrrA M1, or pVrrA M2) were grown in M9 minimal medium. GFP fluorescence was measured, and fluorescence of the control strains was set to 1.

Data information: In (E), data are presented as mean ± SD, $n = 3$.

Source data are available online for this figure.

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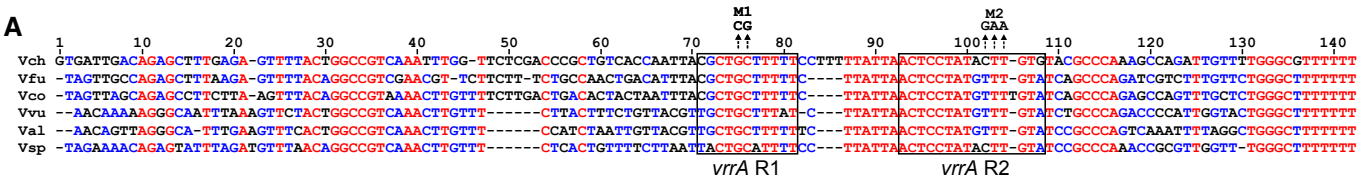
A
M1
CG
M2
GAA
1 10 20 30 40 50 60 70 80 90 100 110 120 130 140
Vch GTGATTGACAGAGCTTTGAGA-GTTTTACTGGCCGTCAAATTTGG-TTCTCGACCCGCTGTCACCAATTACGCTGCTTTTTTCCTTTTTATTAACTCCTATACTT-GTGTACGCCCAAAGCCAGATTGTTTTGGGCGTTTTTT
Vfu -TAGTTGCCAGAGCTTTAAGA-GTTTTACAGGCCGTCGAACGT-TCTTCTT-TCTGCCAACTGACATTTACGCTGCTTTTTC----TTATTAACTCCTATGTTT-GTATCAGCCCAGATCGTCTTTGTTCTGGGCTTTTTTT
Vco -TAGTTAGCAGAGCCTTCTTA-AGTTTACAGGCCGTAAACTTGTTTTCTTGACTGACACTACTAATTTACGCTGCTTTTTC----TTATTAACTCCTATGTTTTGTATCAGCCCAGAGCCAGTTTGCTCTGGGCTTTTTTT
Vvu --AACAAAAAGGGCAATTTAAAGTTCTACTGGCCGTCAAACTTGTTT------CTTACTTTCTGTTACGTTGCTGCTTTAT-C----TTATTAACTCCTATGTTT-GTATCTGCCCAGACCCCATTGGTACTGGGCTTTTTTT
Val --AACAGTTAGGGCA-TTTGAAGTTTCACTGGCCGTCAAACTTGTTT------CCATCTAATTGTTACGTTGCTGCTTTTTTC----TTATTAACTCCTATGTTT-GTATCCGCCCAGTCAAATTTTAGGCTGGGCTTTTTTT
Vsp -TAGAAAACAGAGTATTTAGATGTTTAACAGGCCGTCAAACTTGTTT------CTCACTGTTTTCTTAATTACTGCATTTTCC----TTATTAACTCCTATACTT-GTATCCGCCCAAACCGCGTTGGTT-TGGGCTTTTTTT
vrrA R1
vrrA R2
B
pCtr
pMicV
pMicV M1
pVrrA
pVrrA M1
pVrrA M2
VrrA
MicV
5S rRNA
1 2 3 4 5 6
C
WT ΔrpoE
ΔvrrA ΔrpoE
ΔmicV ΔrpoE
ΔvrrA ΔmicV ΔrpoE
pCtr
pBAD-rpoE
0 15 0 5 10 15 0 15 0 5 10 15 0 15 0 5 10 15 0 15 0 5 10 15
[min]
VrrA
MicV
5S rRNA
1 2 3 4 5 6 7 8 9 10 11 12 1 2 3 4 5 6 7 8 9 10 11 12
D
-24 (M1*) -5
ompT 5'-AUU CG GAU-3'
AAG AAAAGGCAGUG
UUC UUUUUCGUCGC
VrrA 3'-UUU C AUU-5'
86 (M1) 66
E
relative fluorescence [AU]
2.0
1.5
1.0
0.5
0.0
ompT
ompT M1*
pCtr
pVrrA
pVrrA M1
pVrrA M2
pCtr
pVrrA
pVrrA M1

Figure EV2.

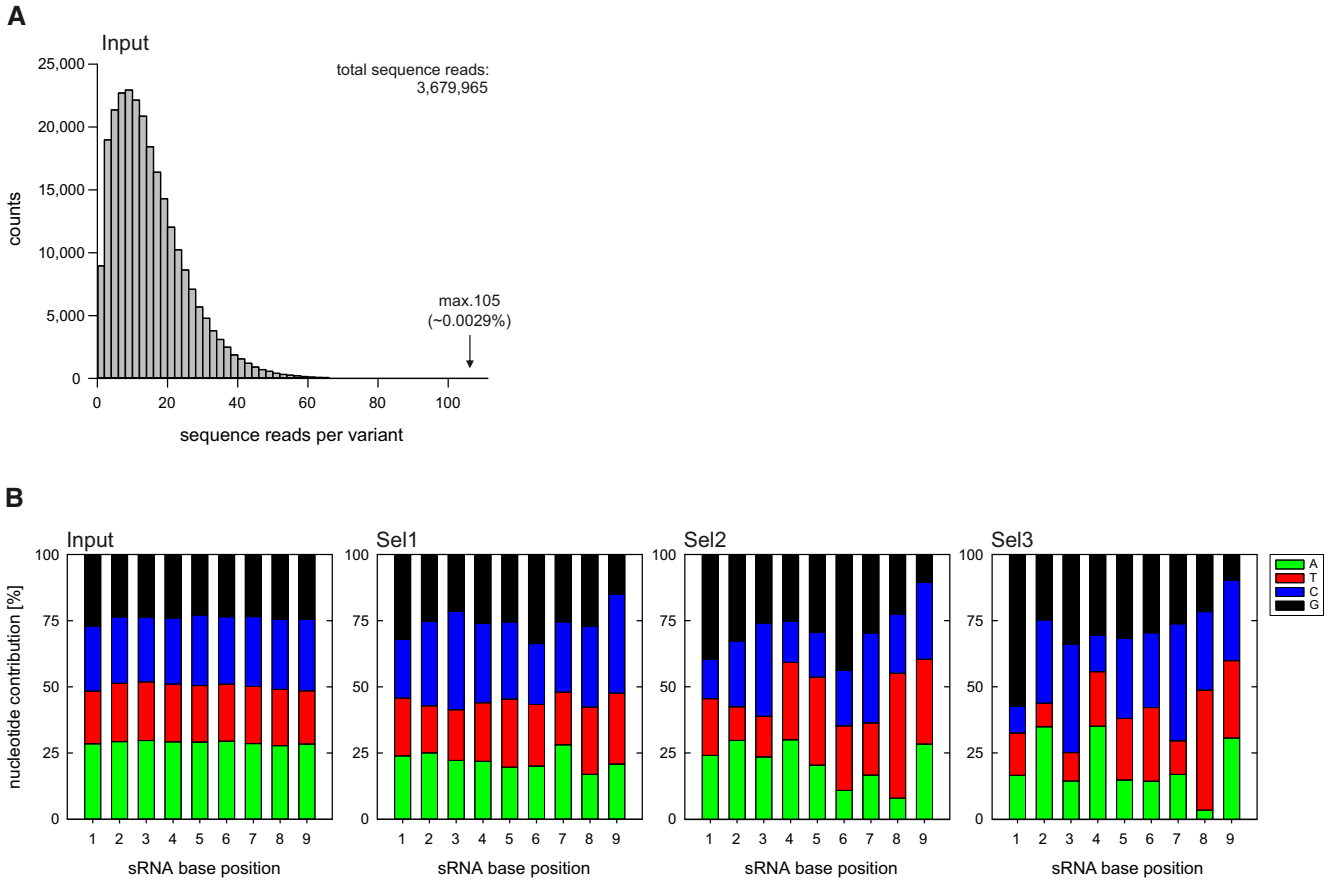

Figure EV3. Synthetic sRNA library composition and nucleotide contributions (related to Fig 5).

A, B A synthetic sRNA library based on a RybB scaffold with nine randomized nucleotides at the 5′ end was cloned into plasmid backbones and transferred into *V. cholerae* Δ*rpoE*. The resulting clones were pooled and treated with ethanol (3.5% final conc.) for 6 h. After treatment, the surviving cells were recovered on agar plates, pooled, and subjected to consecutive rounds of ethanol treatment for a total of three selections. After each selection, plasmid contents of surviving cells were analyzed by high-throughput sequencing. (A) Density histogram depicting the sequence read counts of obtained sRNA variants before ethanol treatment (Input). (B) Nucleotide contributions at the randomized positions in the synthetic sRNA libraries, before ethanol treatment (Input) and after consecutive ethanol treatments (Sel1, 2, 3). A = adenine, T = thymine, C = cytosine, G = guanine.

Data information: In (B), data are presented as mean, $n = 2$.
Source data are available online for this figure.

Figure EV4. Synthetic sRNA variants are enriched in laboratory selection experiments and mediate ethanol resistance (related to Fig 6).

A *Vibrio cholerae* Δ*rpoE* strains carrying the sRNA library before (input) or after consecutive ethanol selection experiments (Sel1, Sel2, and Sel3) were cultivated in LB medium to OD_{600} of 2.0. RNA samples were collected and analyzed for *omp* mRNA levels using qRT–PCR.

B Pie chart indicating the distribution of synthetic sRNA variants after three consecutive ethanol treatments (Sel3). The dashed red line indicates the fraction of the 15 most abundant sequence variants.

C The frequency of the 15 most abundant (top 15) sRNA variants was determined before ethanol treatment (Input) and after consecutive ethanol treatments (Sel1, 2, 3).

D *Vibrio cholerae* wild-type and Δ*rpoE* strains carrying an empty vector control (pCtr), synthetic sRNA expression plasmids (psRNA1-15), *rybB* expression plasmids (pRybB), or expression plasmids containing a *rybB* variant with deletion of nine nucleotides at the 5′ end (pRybBΔ9) were grown to OD_{600} of 0.2. Cells were treated with ethanol (3.5% final conc.) for 5 h. After treatment, the strains were serially diluted (1:10 steps) and spotted onto agar plates.

Data information: In (A, C), data are presented as mean ± SD, $n = 2$.
Source data are available online for this figure.

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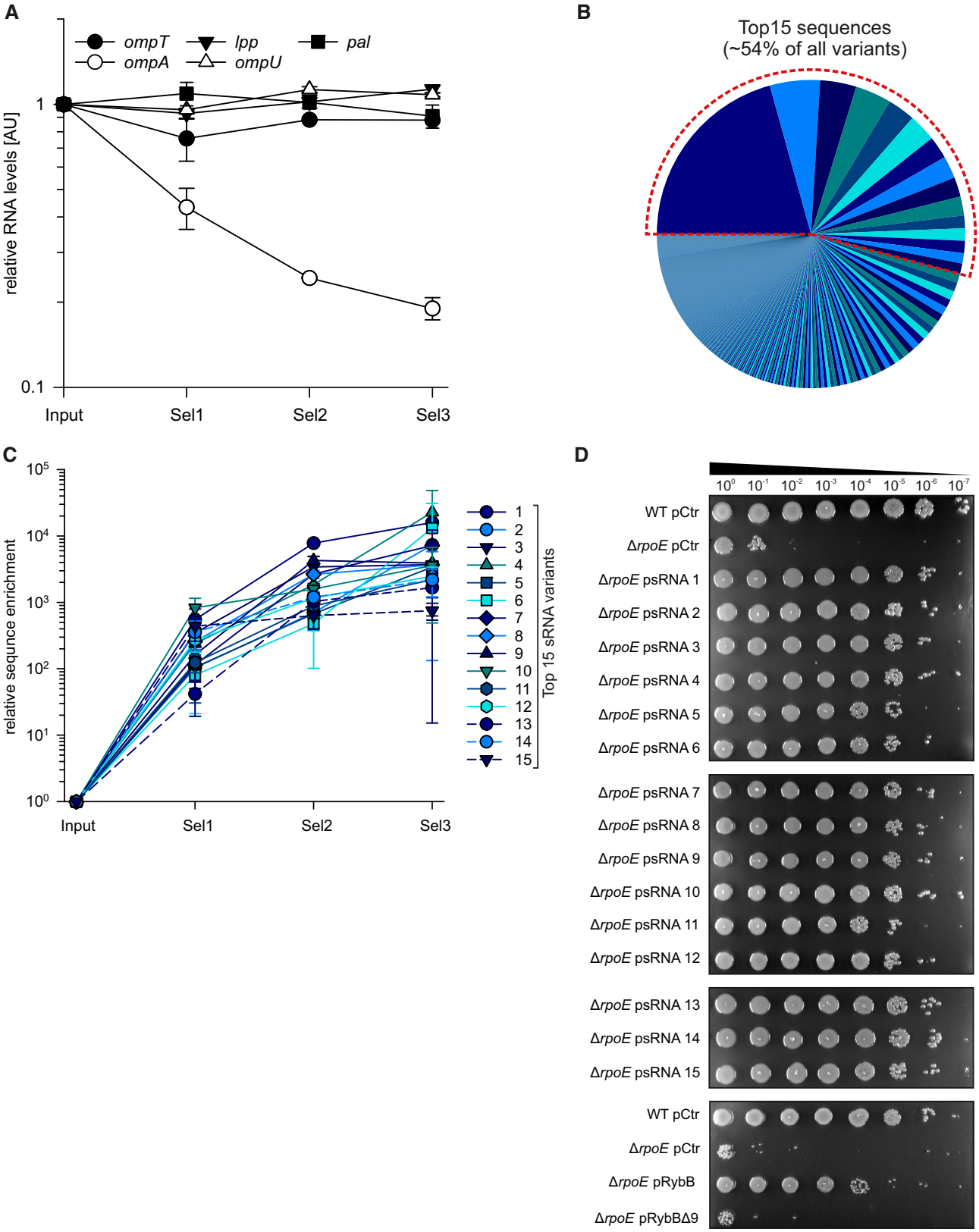

Figure EV4.

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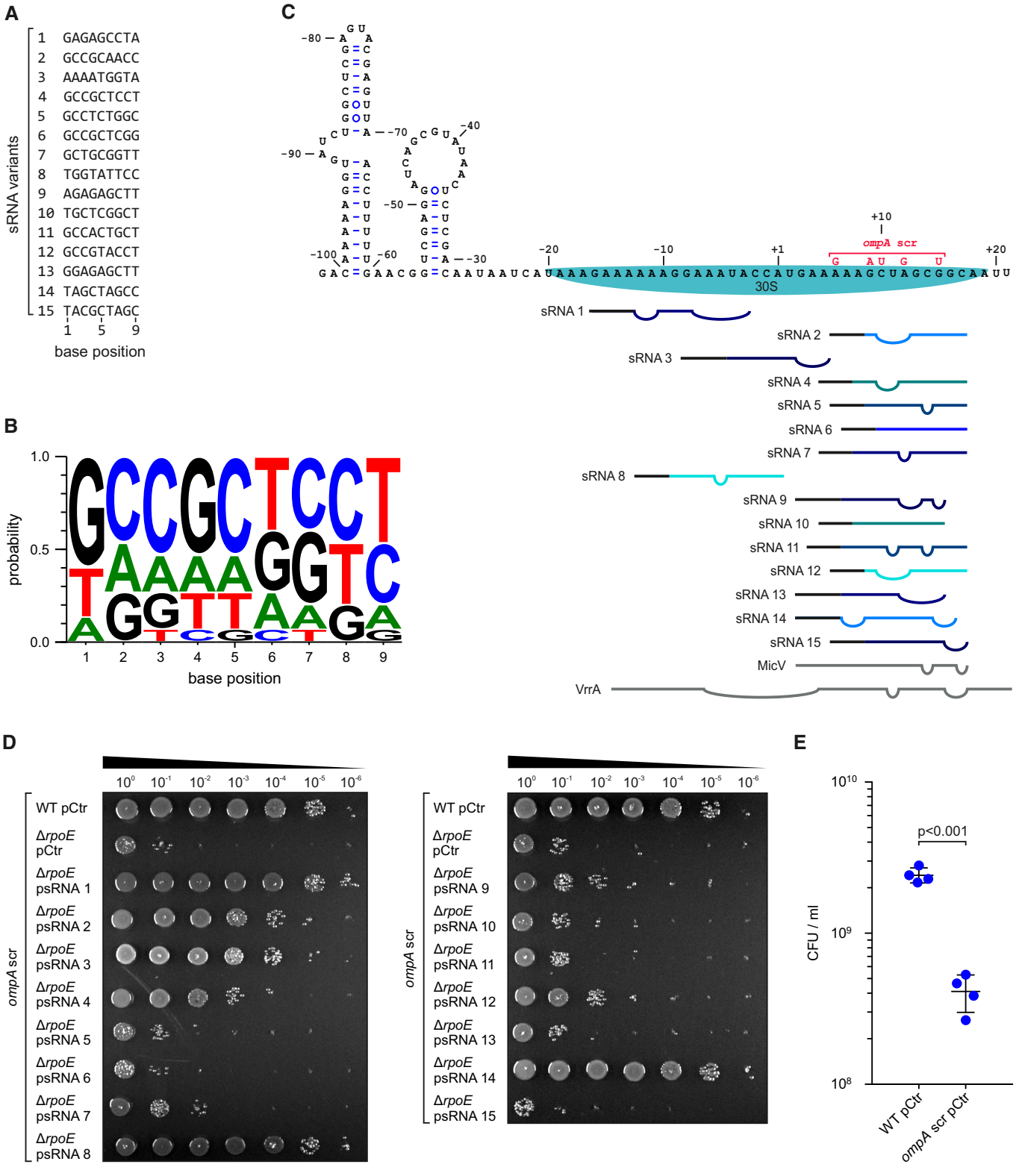

Figure EV5.

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Figure EV5. Base-pairing of enriched sRNA variants to *ompA* mRNA is sufficient to mediate ethanol resistance (related to Fig 6).

A Sequence alignment of the 15 most abundant (top 15) sRNA variants.

B Consensus motif for the top 15 sRNA variants.

C Secondary structure model of *ompA* mRNA including the predicted base-pairing interactions of the top 15 sRNA variants. Straight lines indicate pairing bases, and bulges indicate non-pairing bases. Pairing bases corresponding to the variable region of the variants are depicted in color, and pairing bases corresponding to the backbone are depicted in black. MicV and VrrA are shown in gray. Numbers indicate the position on the *ompA* mRNA relative to the AUG start codon. The predicted position of the 30S ribosomal subunit and the *ompA* scr mutation are indicated.

D *Vibrio cholerae* wild-type *ompA* scr and Δ*rpoE ompA* scr strains carrying an empty vector control (pCtr) or synthetic sRNA expression plasmids (psRNA1-15) were grown to OD_{600} of 0.2. Cells were treated with ethanol (3.5% final conc.) for 5 h. After treatment, the strains were serially diluted (1:10 steps) and spotted onto agar plates.

E *Vibrio cholerae* wild-type and *ompA* scr mutant strains carrying empty vector controls (pCtr) were grown to OD_{600} of 0.2 and challenged with ethanol (3.5% final conc.). After 5 h of treatment, serial dilutions were prepared, recovered on agar plates, and CFU/ml were determined.

Data information: In (E), data are presented as mean ± SD, $n = 4$. Statistical significance was determined using a two-tailed, unpaired Student's *t*-test.
Source data are available online for this figure.

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