# Supplementary information

# MtvR is a global regulatory sRNA in *Burkholderia cenocepacia*

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# Supplementary Methods

**Assessment of anti-MtvR transcripts**

The time required by the anti-MtvR transcript to silence MtvR was assessed using *B. cenocepacia* J2315 transformed with plasmid pCGR16, grown for 24h (t = 0), time after which the expression of anti-MtvR was induced by the addition of 1% of L-arabinose (final concentration) to the culture. Samples were taken after 0, 5 and 10 min. RNA was extracted and processed as described in the main text.

**Bioinformatic analysis**

The MtvR sRNA sequence was aligned with the RNA sequence of the putative mRNA targets, predicted by the sRNATarget software (1), using the RNAHybrid web tool (2). In order to predict potential sigma-factor binding sites in putative MtvR mRNA targets, regulated by $\sigma^{70}$ transcription factor, the BPROM software (Softberry, Inc., Mt. Kisco, NY) bioinformatics tool (minimum score of 7) and MEME suite webserver (3) were used. The alignment of the MtvR encoding sequences from Bcc strains was performed using Clustal W (4).

**EMSA experiments with RNA chaperones**

The ability of 25 nM (final concentration) of MtvR to bind to Hfq (concentrations ranging from 0.1 to 200 nM of the hexameric form of Hfq ($Hfq_6$)), or to Hfq2 (concentrations ranging from 0.1 to 200 nM of the trimeric form of Hfq2 $(Hfq2)_3$), was also evaluated with EMSA assays. Non-labelled yeast tRNA (Ambion) was added in excess to each sample to minimize non-specific binding. Incubation, resolution of RNA-RNA and RNA-protein complexes and detection of band-shifts was performed as previously described (5).

# Supplementary Figures

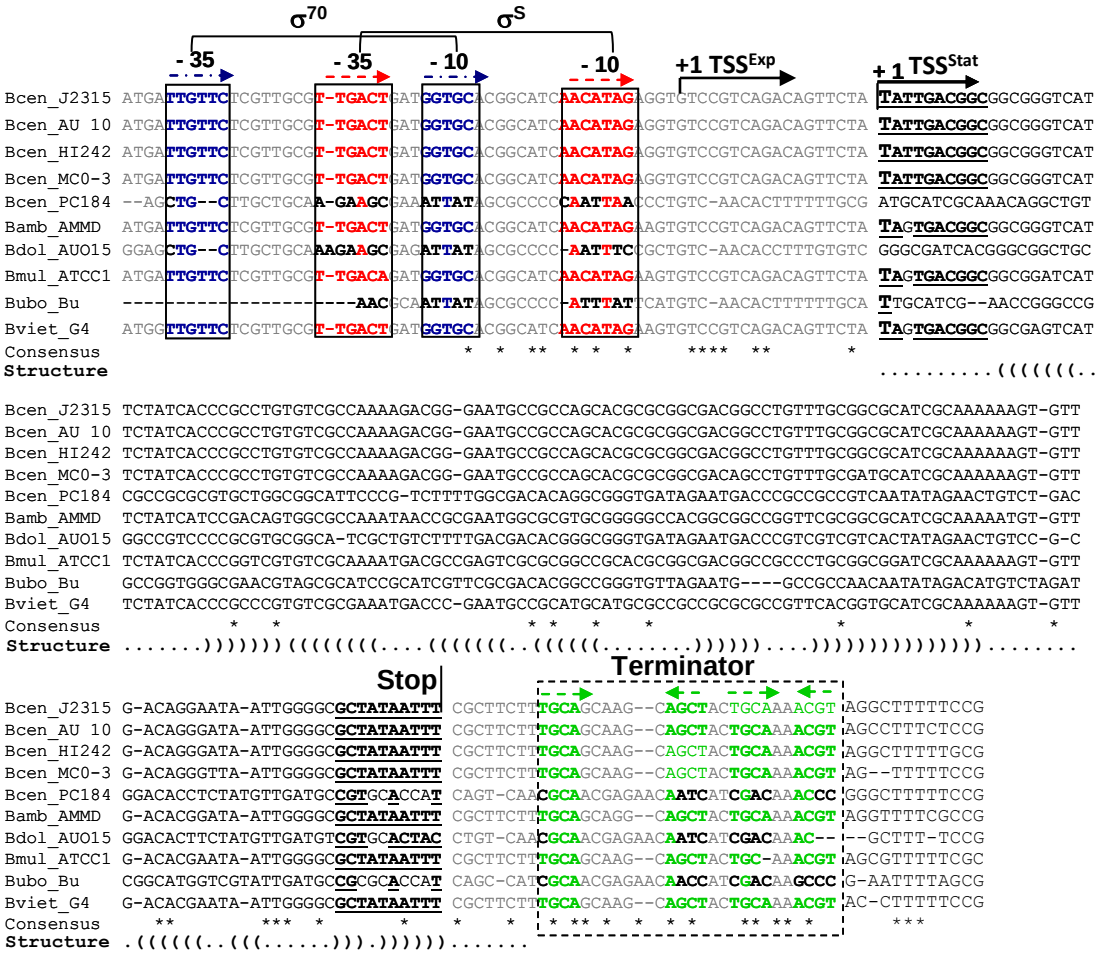


**FIG S1. MtvR is exclusive to Bcc strains.** Sequence alignment of the MtvR homologues of the indicated *Burkholderia* strains. Transcribed sequences are shown in black, non-transcribed elements are shown in grey. The putative $\sigma^{70}$ (blue, p value = $4.84e^{-4}$) and $\sigma^{S}$ (red, p value = $2.37e^{-3}$) regulatory sequences predicted using the MEME suite webserver are indicated. Non conserved nucleotides in regulatory sequences are shown in black. The MtvR Transcription Start Sites (TSS) are represented by capital casing with the conserved nucleotides shown in bold and underlined (exp, exponential; stat, stationary phase). The Transcription Stop Site is indicated by an underlined bold sequence, and the terminator sequences are represented in green, with the non-conserved nucleotides in black. Asterisks below the sequences indicate conserved nucleotides. Bcen, *B. cenocepacia*; Bamb, *B. ambifaria*; Bdol, *B. dolosa*; Bmul, *B. multivorans*; Bubo, B. ubonensis; Bviet, *B. vietnamiensis*.

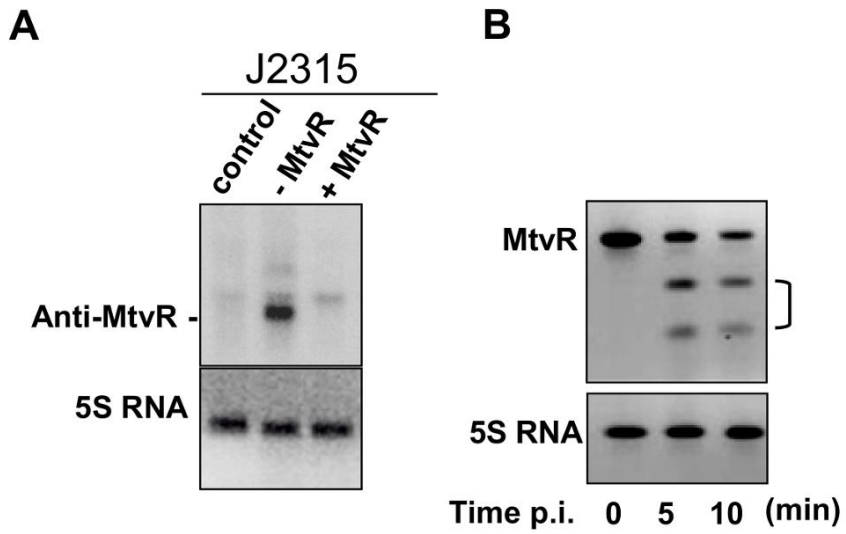


**FIG S2**. ***hfq*** **mRNA levels are affected by MtvR**. A) Northern blot analysis of the transcript designed to silence MtvR in wt, wt overexpressing the anti-MtvR RNA (- MtvR), and wt overexpressing MtvR (+ MtvR). B) Effects of expression of the anti-MtvR on the MtvR levels, after induction with 2% L-arabinose for the indicated time. Putative processing products are marked with bracket.

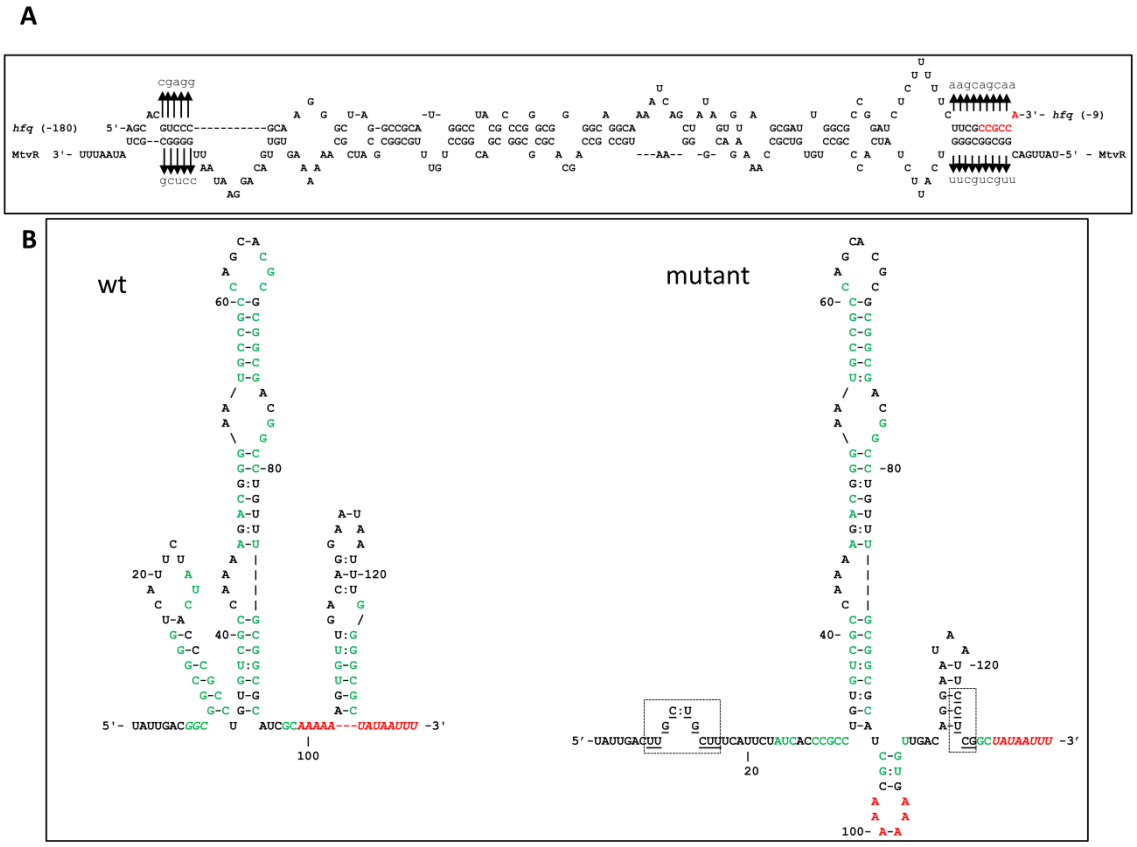


**FIG S3. The MtvR sRNA forms stable duplex with the *hfq* 5'-UTR.** A) Predicted interaction between the *hfq* 5'-UTR and MtvR (red, putative Ribosomal binding site). Mutated nucleotides in the mRNA and sRNA are indicated by arrows, and represented in lower case. Numbers in parenthesis represent nucleotide positions, in reference to the transcription start site. B) Predicted secondary structure of MtvR and of the mutated MtvR version, using Mfold (6). Nucleotides shown in green are putatively involved in base pairing with the *hfq* 5'-UTR. The sequence shown in red is the predicted Hfq binding sequence. The mutated nucleotides are underlined and boxed.

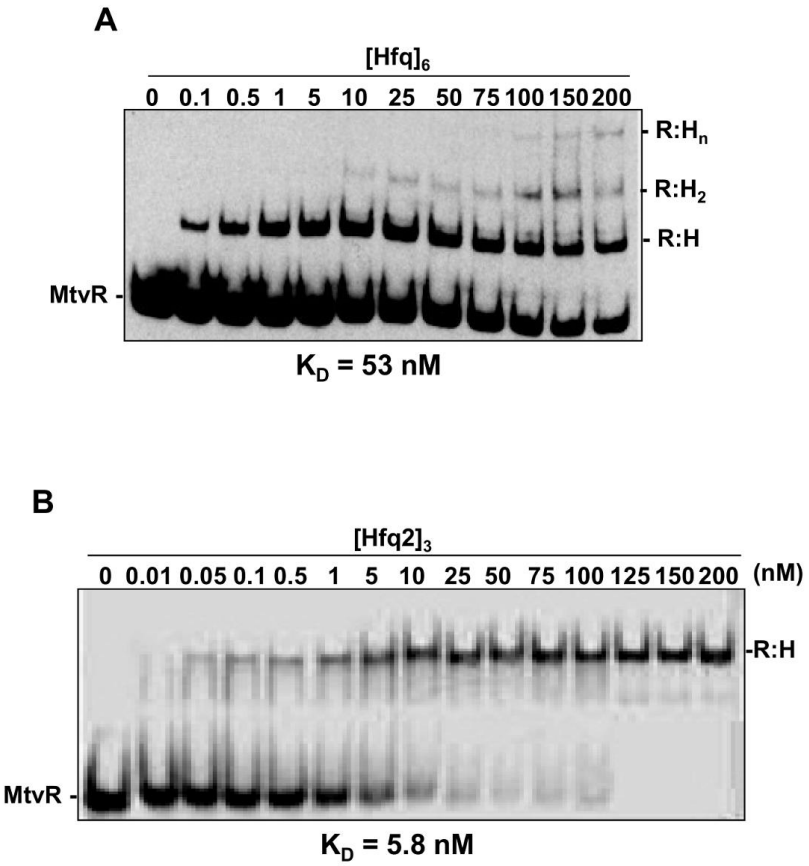


**FIG S4**. **MtvR binds to Hfq and Hfq2**. EMSA assays performed to assess the ability of Hfq (A), or Hfq2 (B) to bind to 25 nM of MtvR. The estimated $K_D$ values are shown. Concentrations of the RNA chaperones were calculated based on their respective multimeric state, hexameric for Hfq and trimeric for Hfq2, respectively.

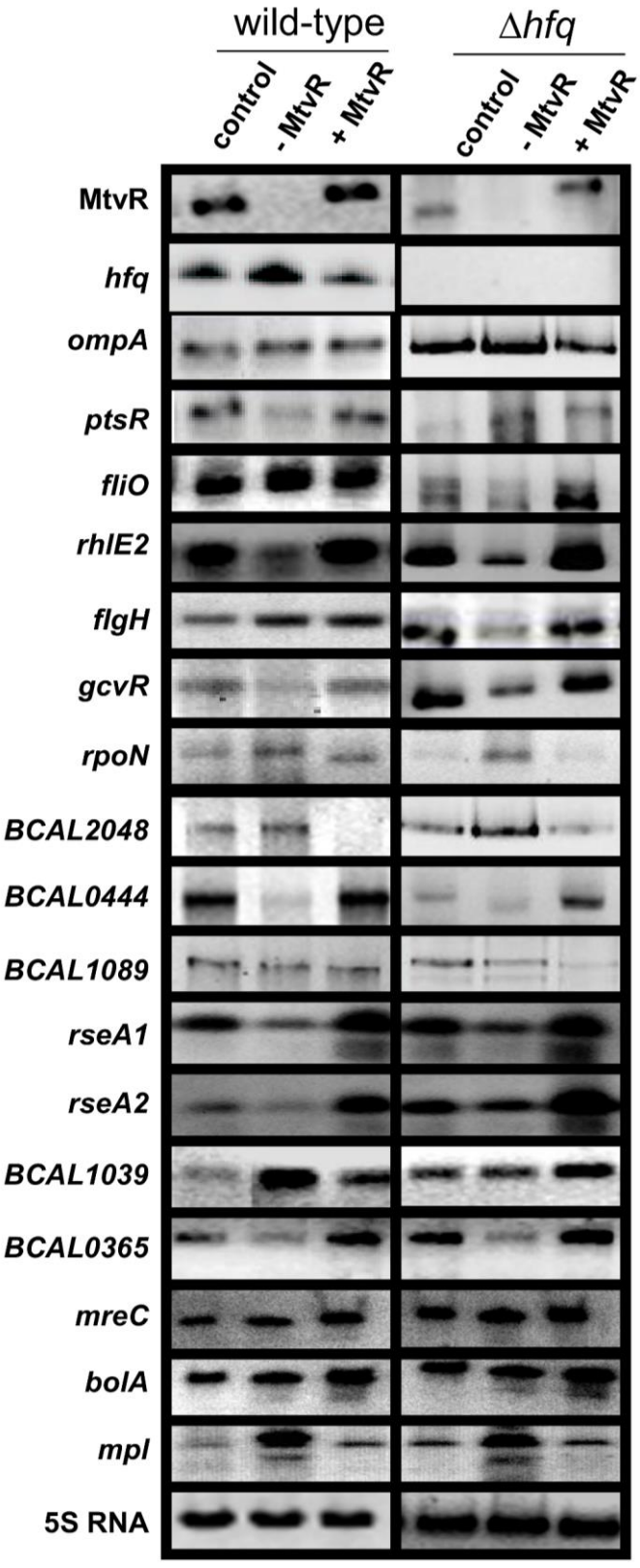


**FIG S5**. **MtvR affects multiple genes**. Northern blot analysis of the effect of MtvR on the mRNA levels of selected predicted targets. Total RNA from the wt (control) and derivative strains with the MtvR silenced (-MtvR), or overexpressing MtvR (+MtvR), and from the Δ*hfq* mutant (CJ1), the CJ1 with MtvR silenced (-MtvR), and the CJ1 overexpressing MtvR (+MtvR) was probed.

# Supplementary Tables

**Table S1**. Bacterial strains and plasmids used in this work.

| Strain or plasmid | Description | Reference or source |
|---|---|---|
| *B. cenocepacia* J2315 | CF sputum isolate | (7) |
| *B. cenocepacia* CJ1 | *B. cenocepacia* J2315 *hfq* mutant | (8) |
| *B. cenocepacia* CJ2 | *B. cenocepacia* J2315 *hfq2* mutant | (8) |
| *B. cenocepacia* J2315+pCGR15 | *B. cenocepacia* J2315 overexpressing MtvR | This study |
| *B. cenocepacia* CJ1+pCGR15 | *B. cenocepacia* J2315 *hfq* mutant overexpressing MtvR | This study |
| *B. cenocepacia* J2315+pCGR16 | *B. cenocepacia* J2315 with the sRNA MtvR silenced | This study |
| *B. cenocepacia* CJ1+pCGR16 | *B. cenocepacia* J2315 *hfq* mutant with the sRNA MtvR silenced | This study |

| Plasmids | | |
|---|---|---|
| pMLBAD | $Tmp^R$; used for inducible gene expression | (9) |
| pBBR1MCS | $Cm^R$; used for constitutive gene expression | (10) |
| pCR 2.1 | $Amp^R$; $Km^R$; used *in vitro* transcription | Invitrogen |
| pCGR4 | pET23a+ with the *hfq* encoding sequence clone | (5) |
| pCGR14 | pCR 2.1 with the 140 bp cDNA fragment corresponding to the mtvR sRNA cloned in the XbaI/HindIII sites, and a $Cm^R$ cassette (obtained by PCR from plasmid 0pKD3) cloned in the HincII site | This study |
| pCGR14.1 | pCGR14 digested and self-ligated in the HaeIII sites | This study |
| pCGR15 | pMLBAD with the 140 bp cDNA fragment corresponding to the MtvR sRNA cloned in the EcoRI/XbaI sites (pBAD promoter control) | This study |
| pCGR16 | pMLBAD with the 140 bp cDNA fragment corresponding to the MtvR sRNA cloned in the XbaI/HindIII sites (pBAB promoter control) | This study |
| pCGR20 | pCR 2.1 with the 140 bp cDNA fragment corresponding to the mtvR sRNA cloned in the XbaI/HindIII sites (T7 promoter control) | This study |
| pCGR24 | pCR 2.1 with the 384 bp cDNA fragment corresponding to the *hfq* 5'-UTR cloned in the XbaI/HindIII sites (T7 promoter control) | This study |

| | | |
|---|---|---|
| pCGR25 | pCR 2.1 with the 609 bp cDNA fragment corresponding to the *hfq* full mRNA (5'-UTR and CDS) with 6 histidine at the C-terminus, cloned in the XbaI/HindIII sites (T7 promoter control) | This study |
| pCGR33 | pMLBAD with the 5'-UTR-*hfq*-LacZ DNA fragment (pBAB promoter disrupted, only replicative) | This study |

**Table S2**. Oligonucleotides and primers used in this work.

| Name | Purpose | Sequence 5' –3' | Source |
|---|---|---|---|
| UF | Cloning mtvR | TTTCTAGATATTGACGGCGGCGGGU | This study |
| LF | Cloning mtvR | TTAAGCTTAAATTATAGCGCCCCAATTA | This study |
| NP | Northern analysis of mtvR | CTATCACCCGCCTGTGTCGCCA | This study |
| OMPA | Northern blot probe for *ompA* | AAAAGCTTGTTTGCCGGAAC | This study |
| PTSR | Northern blot probe for *ptsR* | CGCCTCCAGGTATTCGTACA | This study |
| FLIO | Northern blot probe for *fliO* | ATCTCGACGATCGTCGCGCTTTCCTTC | This study |
| RHLE2 | Northern blot probe for *rhlE2* | TACGGCCGATCCGGTGCACGTAG | This study |
| FLGH | Northern blot probe for *flgH* | CATCGGCATCGGCGGCTGCGCCGTCATC | This study |
| GCVR | Northern blot probe for *gcvR* | CGAGGCGCGGGACGAGCCACGCGGAT | This study |
| RECQ | Northern blot probe for *recQ* | GCGACCTGGTCCTGCATCAGCGCGAT | This study |
| RPON | Northern blot probe for *rpoN* | GATCAGTGCGTGCGCGTCGCGCA | This study |
| RPSL | Northern blot probe for *rpsL* | GCCGCGACGCTGGGGGCAGTCCTGCAGGG | This study |
| 2048 | Northern blot probe for *BCAL2048* | TTCGGCTTCGACACGAACGTGCCCTT | This study |
| 0444 | Northern blot probe for *BCAL0444* | CGCCTCCAGGTATTCGTACA | This study |
| 1089 | Northern blot probe for *BCAL1089* | CTCGGTGTGCTGGTTCGTGCGGATC | This study |
| HFQ | Northern blot probe for *hfq* | AAAGGGCAATTGTTACAAG | (8) |
| CGRO105 | Northern blot probe for *rseA2* | TGCGGTAAACGCGACGCTCA | This study |
| CGRO106 | Northern blot probe for *rseA1* | GGTGGTAATGGGCCCACGCA | This study |

| | | | |
|---|---|---|---|
| CGRO107 | Northern blot probe for *BCAL1039* | CGGCGGATGTAGCGGAACAGG | This study |
| CGRO108 | Northern blot probe for *BCAL0365* | GCAGTGACACGAATCGGCGGG | This study |
| CGRO109 | Northern blot probe for *BCAL0640* | GATCCCGCGCACAGCAGCAT | This study |
| CGRO110 | Northern blot probe for *mpl* | GTGGTCGTCTTGCCGTGCGT | This study |
| CGRO111 | Northern blot probe for *mreC* | TGGTTGGCCTGCGTGGACAG | This study |
| CGRO112 | Northern blot probe for *bolA* | GCACGGTGAGCGACAGCGG | This study |
| CGRO113 | MtvR mutagenesis | TTTCTAGATATTGACTTGCTGCTTTATCACCC | This study |
| CGRO114 | MtvR mutagenesis | TTAAGCTTAAATTATAGCCGAGGAATTA | This study |
| CGRO115 | *hfq* mutagenesis | TTTCTAGAGCACCGAGGGCAAGGGCTAG | This study |
| CGRO116 | *hfq* mutagenesis | TTAAGCTTTTGGCTGGCTTAAAA | This study |
| CGRO121 | Fwd primer *hfq* 5'-UTR | TTGGATCCATTGGACGAGGCTTCCGC | This study |
| CGRO122 | Rev primer *hfq* 5'-UTR | TTGGATCCCATGGCGTACTCCATCTTTT | This study |
| CGRO123 | Fwd primer *hfq* his-tag | TTTCTAGAGCACGTCCCGCAAGGGCTAG | This study |
| CGRO124 | Rev primer *hfq* his-tag | TTGGATCCATTGTGGTGGTGGTGGTGGGACGAGGCTTCCGC | This study |
| M13FWD | LacZ amplification | GTAAAACGACGGCCAGT | Invitrogen |
| M13REV | LacZ amplification | AGCGGATAACAATTTCACACAGGA | Invitrogen |
| 5S | Northern blot probe for 5S rRNA | TTCGGGATGGGAAGGGGTGGGA | (8) |
| 5R-MT | 5' RACE experiments | ATACGGGGCTGCGAGAGTCGT | This study |

**Table S4**. Potential MtvR targets in *B. cenocepacia* J2315 genome

| Gene | Function | Locus Tag |
|---|---|---|
| *hfq* | RNA chaperone and Pleiotropic regulator | BCAL1879 |
| *recQ** | ATP-dependent DNA helicase RecQ | BCAL0228 |
| *rpoN* | Nitrogen limitation response transcription regulator | BCAL0813 |
| *rpsL** | Ribosomal subunit involved in translational accuracy. Important for ribosome structure | BCAL0229 |
| *flgH* | The flagellar L-ring protein precursor protects the motor/basal body from shearing forces during rotation | BCAL0570 |
| *rhlE2* | ATP-dependent RNA helicase | BCAL2412 |
| *fliO* | Essential component of the flagellum-specific protein export apparatus | BCAL3504 |
| *gcvR* | *gcvA* LysR family transcriptional regulator | BCAL0382 |
| *BCAL2048* | GntR regulator of a transport cluster (Fe-S), MFS type | BCAL2048 |
| *BCAL0444* | GntR regulator of a transport cluster, ABC type | BCAL0444 |
| *ptsR* | PTS regulator | BCAL0777 |
| *BCAL1089* | AnsC regulator of a ABC transport cluster | BCAL1089 |
| *rseA1* | $\sigma^E$ negative regulatory protein 1 | BCAL2871 |
| *rseA2* | $\sigma^E$ negative regulatory protein 2 | BCAL0999 |
| *BCAL1039* | ATP-binding cassette transporter protein | BCAL1039 |
| *BCAL0365* | Major facilitator Superfamily transporter protein | BCAL0365 |
| *mpl* | UDP-N-acetylmuramate:L-alanyl-gamma-D-glutamyl-meso-diaminopimelate ligase | BCAL3416 |
| *mreC* | Rod-shape determining protein | BCAL0481 |
| *bolA* | Cell shape | BCAL1984 |

* mRNA predicted MtvR targets which mRNA levels were not affected by MtvR.

## Table S5: Hybridization prediction for MtvR and its predicted mRNA targets

```
TARGET : recQ
mfe: -57.4 kcal/mol

target 5'            G                  AA              A    U              A     U           AU   CC      C   UC             AU     A  AA        3'
                AGCG        GU   UGUUAA       UUGCGGU C UCGC   UAG CCGUC CC    GU CU     CAUUC  GUC     GCG C C           GAA  ACUCG CG  G
                UCGC        UA   ACAGUU       AACGCUA G GGCG   GUC GGCAG GG    CA GA     GUAAG  CAG     CGC G G           CUU  UGGGC GC  C
 sRNA  3' UUUAAUA    GGGGUUAA  AGG      GUGAAAA       C C    UUU   C     C  CGCG  C  CCGCC     GG   AAAAC   U U UCCGCCCACUAU   AC     G  GG AGUUAU 5'

TARGET : rpoN
mfe: -64.0 kcal/mol

target 5'                                                          G A  UU AA         UUUCAU    A     GAG     AGUC    C   A UUUGCCAC       A G   A     G  3'
                                                        GC GACAGGC G  G  G   G GCUGGC      GGCA     G      GG    AUAC GGC G          GACCC G CGC  UUGA
                                                        CG UUGUCCG C  C  C   C CGACCG      CCGU     C      CC    UGUG CCG C          CUGGG C GCG  AGUU
 sRNA  3' UUUAAUAUCGCGGGGUUAAUAAGGACAGUUGUGAAAAAACGCUACGCGG  U       G AG GG GCG A               AAGGG AGAAAA  GC      U   C CACUAUCUUA       G   GC    AU 5'

TARGET : rpsL
mfe: -44.7 kcal/mol

target 5'                                                                  AAAUC     U   GA  UU   CAAUUU         AAUAAAC          AA     A    C  3'
                                                                 GCU UUGCC CG      GGCG GCA  UU  GUU           CAGG          AAUG CC     CC UCAA
                                                                 CGG AGCGG GC      CCGC CGU  AG  CAG           GUCC          UUAC GG     GG AGUU
 sRNA  3' UUUAAUAUCGCGGGGUUAAUAAGGACAGUUGUGAAAAAACGCUACGCGGCGUUUGUC   C     C  GCACGA        A   GG   AAAACCGCUGU    GCCCACUAUC    U  GCGGC  C    AU 5'

TARGET : flgH
mfe: -66.3 kcal/mol

target 5'             G   A              A          AAGA  U   C  AA                 C                A                C    C  C       C  3'
                    CU CAG     CC GUC ACGC        GAUG    GC AAC GG  GUC CCGC CGU     CG CA            UGA GCAGGU          U GCCU CC UCGUCAG
                    GG GUU     GG CAG UGUG        CUAC    CG UUG CC  CAG GGCG GCA     GC GU            GCU UGUCCG          A UGGG GG GGCAGUU
 sRNA  3' UUUAAUAUCGC  G   AAUAA  A   U    AAAAAACG    GCGG  U   U  GG   C    C   CGACC  C  AAGGGCAGAAAACC   G      CCCACUAUCUU C    C  C       AU 5'

TARGET : rhlE2
mfe: -65.4 kcal/mol

target 5'                                                    G  ACAGAAU  GACA           G    CAAAA                                                3'
                                                    CGUCG   ACGGGCU  UG CG       CG    UGGUGGCGUUUC   CU      GGCGC GGCGGG
                                                    GCGGC   UGUCCGG  GC GC       GC    ACCGCCGUAAGG   GA      CUGUG CCGCCC
 sRNA  3' UUUAAUAUCGCGGGGUUAAUAAGGACAGUUGUGAAAAAACGCUAC     GUU       CA  G  GC       ACG             GCA  AAACCG     U      ACUAUCUUACUGGGCGGCGGCAGUUAU 5'

TARGET : fliO
mfe: -64.7 kcal/mol

target 5'                                        C    G   C   C          G  U  A     A     AU            U   CUUCCG    C    UU G    G         3'
                                                  GCGC GCA ACA GCUG  GCCG GC UG    CG CGUUC           CGAU CAG      GAUA    G  C GCCG C
                                                  CGCG CGU UGU CGGC  CGGC CG AC    GC GUAAG           GCUG GUC      CUAU    C  G CGGC G
 sRNA  3' UUUAAUAUCGCGGGGUUAAUAAGGACAGUUGUGAAAAAACGCUA    G   U   C    AG    G  C  GACC  C     GGCAGAAAACC    U   CGCCCA    CUUA UG G    G CAGUUAU 5'
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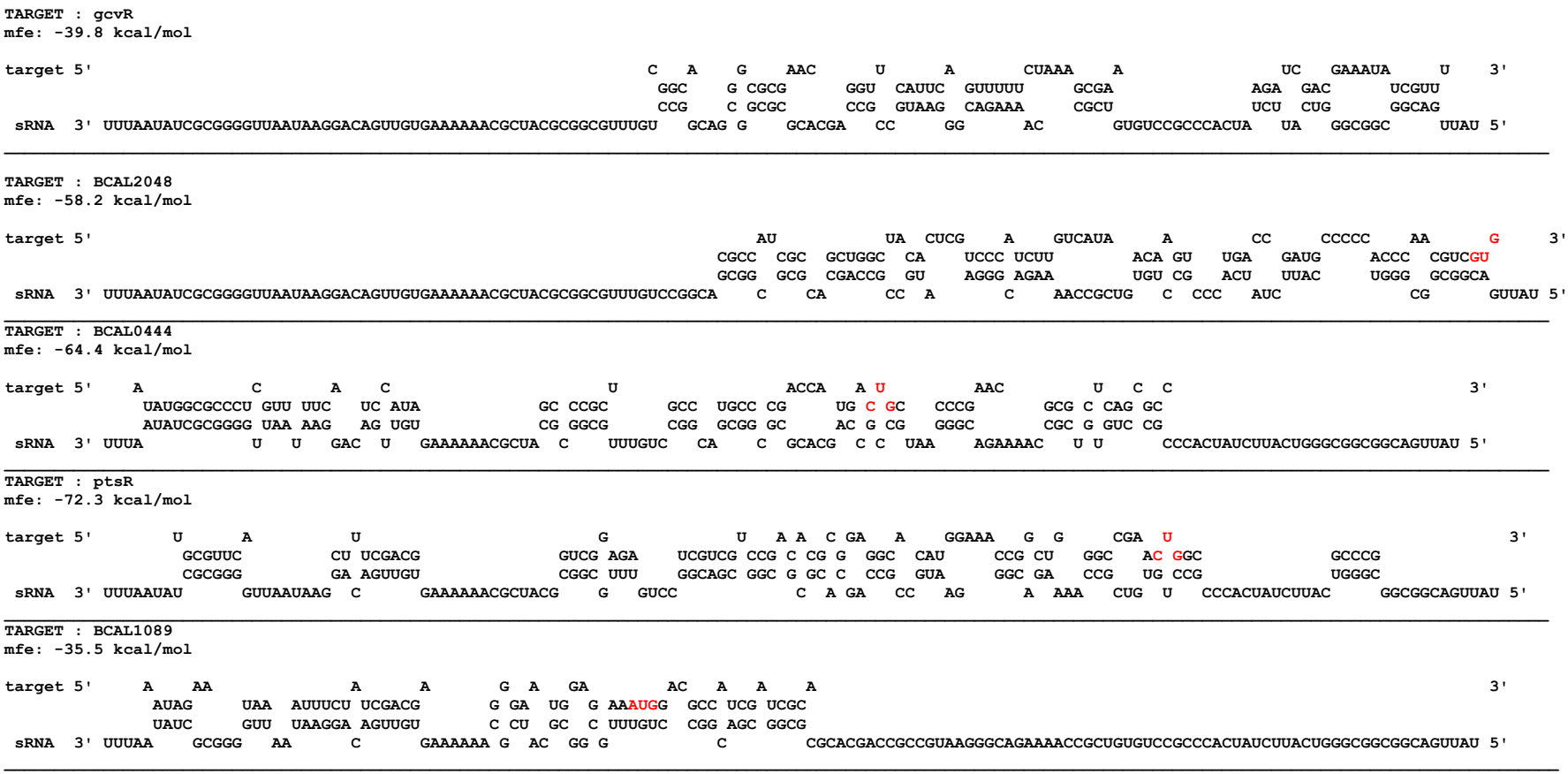


Alignment of the MtvR sRNA sequence with the mRNA targets tested. mfe: minimum folding energy; the putative start codon in highlighted in red lettering.

**Table S6**: Relative fold-change of MtvR putative mRNA targets

| | Wild-type | | Δ*hfq* | | Wild-type vs Δ*hfq* | | |
|---|---|---|---|---|---|---|---|
| | - MtvR | + MtvR | - MtvR | + MtvR | MtvR | - MtvR | + MtvR |
| MtvR | ND | + 1.3 | - 6.3 | + 1.4 | + 2.6 | - 20.4 | + 1.5 |
| *hfq* | + 1.5 | - 6.9 | ND | ND | ND | ND | ND |
| *ompA* | NC | NC | NC | NC | - 2.0 | - 1.9 | - 1.3 |
| *ptsR* | - 2.0 | NC | - 3.0 | - 2.6 | + 2.9 | - 1.7 | NC |
| *fliO* | NC | NC | - 1.5 | + 2.4 | + 2.7 | + 3.9 | + 1.3 |
| *rhlE2* | - 1.5 | + 1.4 | - 1.3 | NC | NC | NC | NC |
| *flgH* | - 1.3 | + 1.3 | - 1.9 | NC | - 1.4 | NC | NC |
| *gcvR* | - 1.6 | NC | - 2.7 | NC | - 1.7 | NC | - 1.5 |
| *rpoN* | + 1.5 | NC | + 3.7 | - 1.2 | + 2.2 | NC | + 2.4 |
| *BCAL2048* | + 1.3 | - 7.1 | + 1.3 | - 1.5 | - 1.3 | NC | - 4.2 |
| *BCAL0444* | - 3.8 | NC | - 2.0 | + 2.4 | + 6.2 | + 3.0 | + 2.0 |
| *BCAL1089* | - 1.2 | NC | - 1.5 | - 3.7 | - 1.6 | NC | + 1.7 |
| *rseA1* | - 1.8 | + 1.2 | - 2.0 | NC | + 1.2 | + 1.2 | NC |
| *rseA2* | - 1.9 | + 3.7 | - 1.2 | + 1.7 | - 2.3 | - 2.8 | NC |
| *BCAL1039* | + 3.6 | NC | NC | + 1.2 | - 1.4 | NC | - 1.5 |
| *BCAL0365* | - 1.6 | + 1.9 | - 2.6 | + 3.5 | - 1.7 | NC | NC |
| *mreC* | + 1.6 | + 1.6 | - 1.2 | NC | - 1.9 | NC | NC |
| *bolA* | NC | + 1.2 | NC | NC | NC | NC | NC |
| *mpl* | + 4.8 | + 1.5 | + 3.1 | NC | NC | NC | NC |

ND, not determined; NC, no fold-change (NC: -1.2 < FC < 1.2; $p \leq 0.05$), SD < 0.01 FC.

## Supplementary References


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