SUPPLEMENTAL FIGURE LEGENDS-

Fig S1. CbsR12 is processed by RNase III. (**A**). CbsR12 secondary structure as predicted by mFold. Nucleotide 1 was determined to be the TSS for the full-size transcript by 5' RACE. Red asterisks indicate apparent alternative TSSs by 5' RACE. Dotted line indicates the putative RNase III processing area. Blue solid lines indicate consensus CsrA-binding sites. (**B**). RNase III assay of *in vitro*-transcribed CbsR12 with the *C. burnetii* IVS RNA as a positive control. Results from treatment with *E. coli* (*Ec*) RNase III (New England BioLabs), recombinant *C. burnetii* (*Cb*) RNase III or no-enzyme controls are shown and done as previously described (1). Arrows indicate RNase III-processed (blue) and un-processed (red) CbsR12 RNA.

Fig S2. Location of the MB-*cbsR12* transposon insertion to inactivate *cbsR12*. (**A**). The *cbsR12* gene and promoter elements are highlighted by the indicated colors, while the location of the *Himar* transposon insertion producing the MB-*cbsR12* strain is marked by a black arrow. Red arrows denote primer-binding sites for PCR confirmation of the lesion (forward and reverse primers above and below their annealing sequences, respectively). (**B**). PCR products confirming transposon insertion in *cbsR12* of MB-*cbsR12* (red arrow) by loss of the ~250 bp amplicon and reintroduction of *cbsR12* in MB-*cbsR12*-Comp (blue arrow). (**C**). Copy number qPCR analysis confirming a single additional insertion of *cbsR12* in the MB-*cbsR12*-Comp strain. Values represent the means ± standard error of means (SEM) of three independent determinations.

Fig S3. Additional biological replicates for ACCM-2 and THP-1 growth curves. Growth curves for MB-WT, MB-*cbsR12*, and MB-*cbsR12*-Comp strains in ACCM-2 (**A, B**) or THP-1 cells (**C, D**) as determined by qPCR. The 0dpi time point refers to the inoculum. Values represent means ± standard error of means (SEM) of three technical replicates.

Fig S4. CbsR12 competitively binds *carA*, *metK*, and *cvpD* transcripts in a dose-dependent manner. RNA-RNA EMSAs showing hybridization reactions between biotin-labeled CbsR12 (Bio-CbsR12) and an *in vitro*-transcribed segment of *carA* (**A**), *metK* (**B**), or *cvpD* (**C**). Anti-CbsR12 represents a positive control consisting of a transcript equal in size but antisense to the CbsR12 transcript. A cold-chase sample containing Bio-CbsR12 + un-labeled CbsR12 + CarA/MetK/CvpD shows competitive (specific) binding relative to Bio-CbsR12 plus target alone, while increasing the dose of *carA*/*metK*/*cvpD* transcript (from 2 nM to 10 nM) increases the amount of retarded sample signal on the blot. Arrows indicate un-bound Bio-CbsR12 (blue) and Bio-CbsR12 bound to its RNA targets (red).

Fig S5. Artemis views of CbsR12 binding to *carA*, *metK*, *ahcY*, and *cvpD* transcripts. Artemis representation of Crosslink-Seq results for MB-WT. Red and blue lines represent the two biological replicates. (**A**). CbsR12 crosslinking with *carA* reads (blue arrow). (**B**). CbsR12 crosslinking with *metK* reads (blue arrow) and *ahcY* reads (red arrow). (**C**). CbsR12 crosslinking with *cvpD* reads (blue arrow).

Fig S6. CbsR12 downregulates the quantity of transcripts arising from the 5' end of *cvpD* in LCVs from infected THP-1 cells. (**A**). *cvpD* gene sequence from the 5' TSS to the predicted downstream alternative start codon. Indicated colors highlight the TSSs, the CbsR12-binding site, the putative downstream promoter, putative RBSs, and start codons. Red arrows show primer annealing regions for qRT-PCR (forward and reverse primers above and below their

respective annealing sequences). (**B**). Representation of CbsR12 binding to the *cvpD* transcript as determined by IntaRNA with base numbers indicated. The top strand in the model represents the *metK* sequence, while the bottom strand represents the complementary CbsR12 sequence. (**C**). qRT-PCR of the 5' end of *cvpD* from MB-WT, MB-*cbsR12*, and MB-*cbsR12*-Comp LCVs (3dpi) and SCVs (7dpi) infecting THP-1 cells. Values represent means ± standard error of means (SEM) of three independent determinations (** = $P < 0.01$, one-way ANOVA, *** = $P < 0.001$, one-way ANOVA).

Fig S7. Coomassie blue-stained SDS-PAGE gels corresponding to Fig. 9 to demonstrate loading consistency. (**A**). Proteins (30 µg total) from MB-WT, MB-*cbsR12*, and MB-*cbsR12*-Comp LCVs (mid-log phase; 96h for MB-WT and MB-*cbsR12*-Comp and 144h for MB-*cbsR12*) grown in ACCM-2 were resolved on a 10-20% acrylamide gradient SDS-PAGE gel and stained with Coomassie brilliant blue R. (**B**). Proteins (60 µg total) from MB-WT, MB-*cbsR12*, and MB-*cbsR12*-Comp LCVs (mid-log phase; 96h for MB-WT and MB-*cbsR12*-Comp and 144h for MB-*cbsR12*) grown in ACCM-2 were resolved on a 10-20% acrylamide gradient SDS-PAGE gel and stained with Coomassie brilliant blue R.

Fig S8. CbsR12 in *E. coli* leads to an autoaggregative phenotype and biofilm formation. (**A**). Overnight cultures of *E. coli* Top10 F' harboring pBEST + *carA*5'UTR or pBEST + *carA*5'UTR + *cbsR12* were inoculated into 3 mL LB supplemented with ampicillin (100 µg/mL), grown for 2 h at 37ºC with shaking, then induced with 1 mM IPTG for 3 h before photography. The red arrow indicates autoaggregation of *E. coli* in the presence of CbsR12. (**B**). *in vitro* biofilm formation assay of *E. coli* Top10 F' harboring pBEST or pBEST + *carA*5'UTR + *cbsR12*. Crystal violet staining is indicative of adherence due to biofilm induction. Values represent the average OD_{570} readings of 10 wells ± standard error of means (SEM) of three independent determinations (* = $P < 0.05$, student's *t* test, ** = $P < 0.01$, student's *t* test).

Fig S9. CbsR1 is an additional *C. burnetii* sRNA with RsmY/Z-like characteristics. (**A**). The *cbsR1* gene, predicted promoter elements, and putative LetA-binding site are highlighted by the indicated colors. (**B**). CbsR1 secondary structure as predicted by mFold. Nucleotide 1 was predicted to be the TSS for the full-size transcript by analysis of RNA-Seq datasets. Blue solid lines indicate putative CsrA-binding sites.

Fig S10. Strains, plasmids, and primers used in the study.

SUPPLEMENTAL LITERATURE CITED-

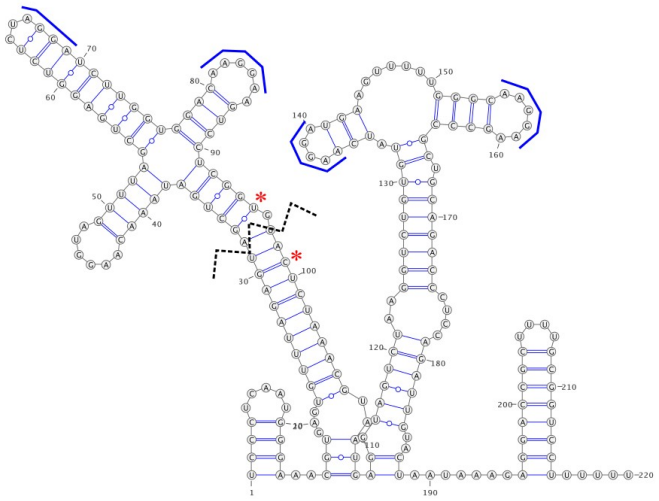
1. Warrier I, Walter MC, Frangoulidis D, Raghavan R, Hicks LD, Minnick MF. 2016. The Intervening Sequence of *Coxiella burnetii*: Characterization and Evolution. *Front Cell Infect Microbiol* **6**: 83.

2. Martinez E, Cantet F, Fava L, Norville I, Bonazzi M. 2014. Identification of OmpA, a *Coxiella burnetii* Protein Involved in Host Cell Invasion, by Multi-phenotypic High-content Screening. *PLoS Pathog* **10**:e1004013.

3. Choi KH, Gaynor JB, White KG, Lopez C, Bosio CM, Karkhoff-Schweizer RR, Schweizer HP. 2005. A Tn7-based broad-range bacterial cloning and expression system. *Nat Methods* **2**:443-448.

Fig. S1

A



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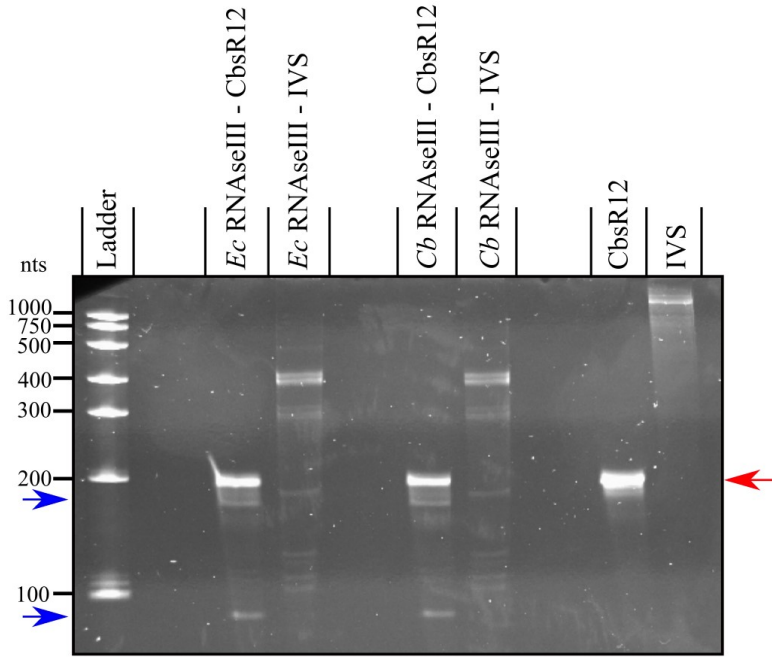
Ladder
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Ec RNAseIII - IVS
Cb RNAseIII - CbsR12
Cb RNAseIII - IVS
CbsR12
IVS
nts
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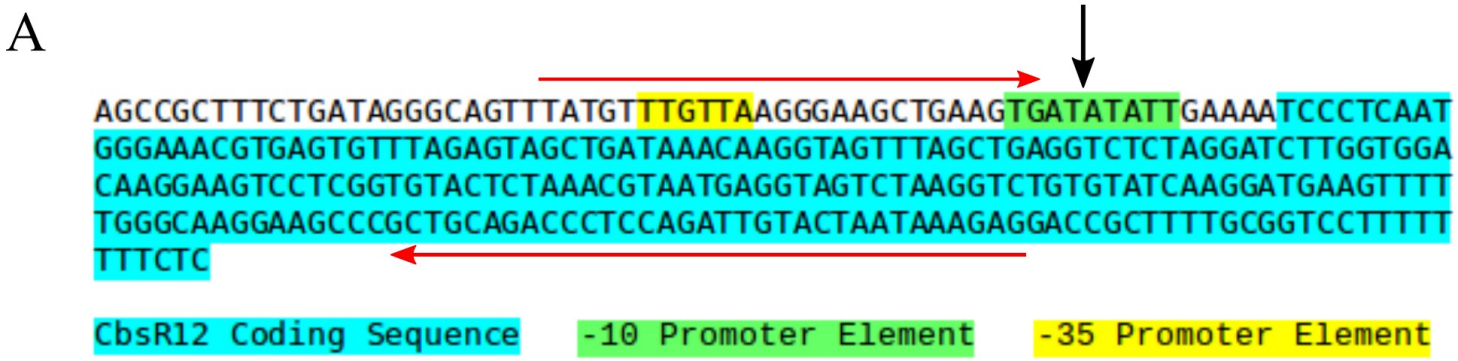
750
500
400
300
200
100

Fig. S2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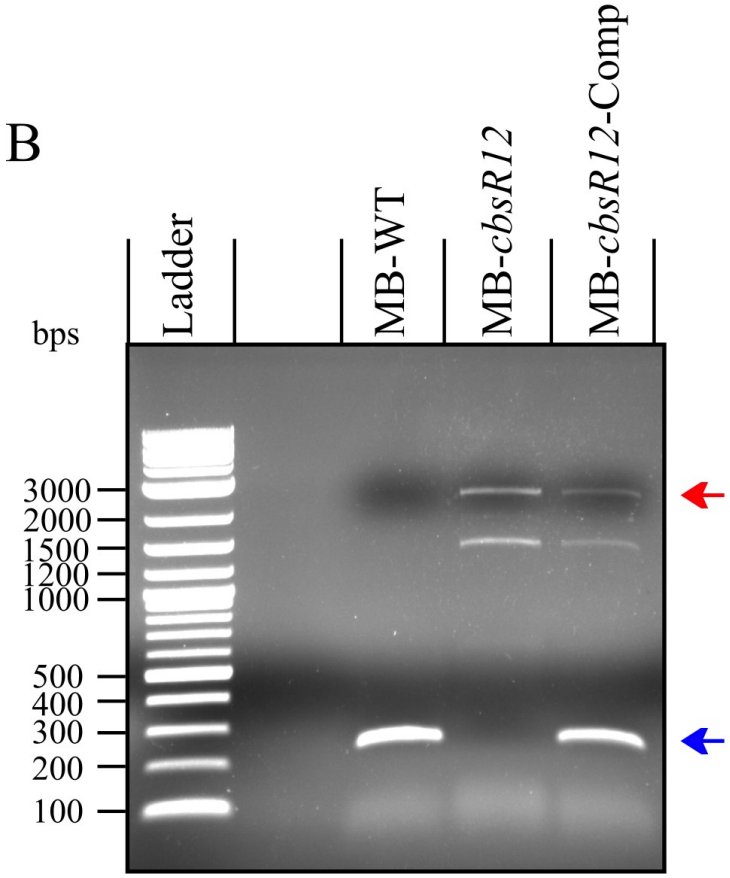
TGGGCAAGGAAGCCCGCTGCAGACCCTCCAGATTGTACTAATAAAGAGGACCGCTTTTGCGGTCCTTTTT
TTTCTC
CbsR12 Coding Sequence
-10 Promoter Element
-35 Promoter Element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MB-cbsR12-Comp
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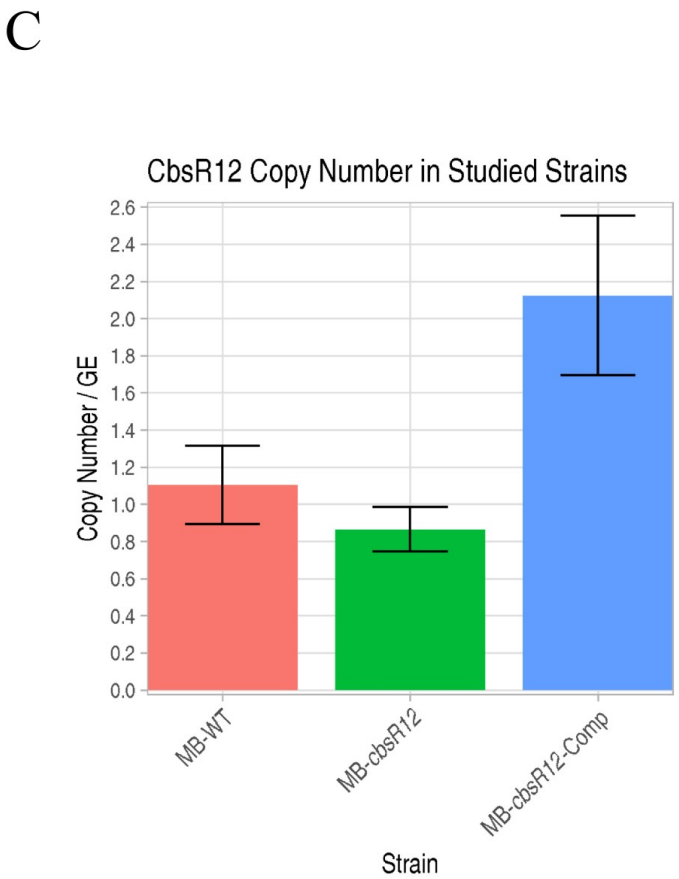
2000
1500
1200
1000
500
400
300
200
100


C
CbsR12 Copy Number in Studied Strains
Copy Number / GE
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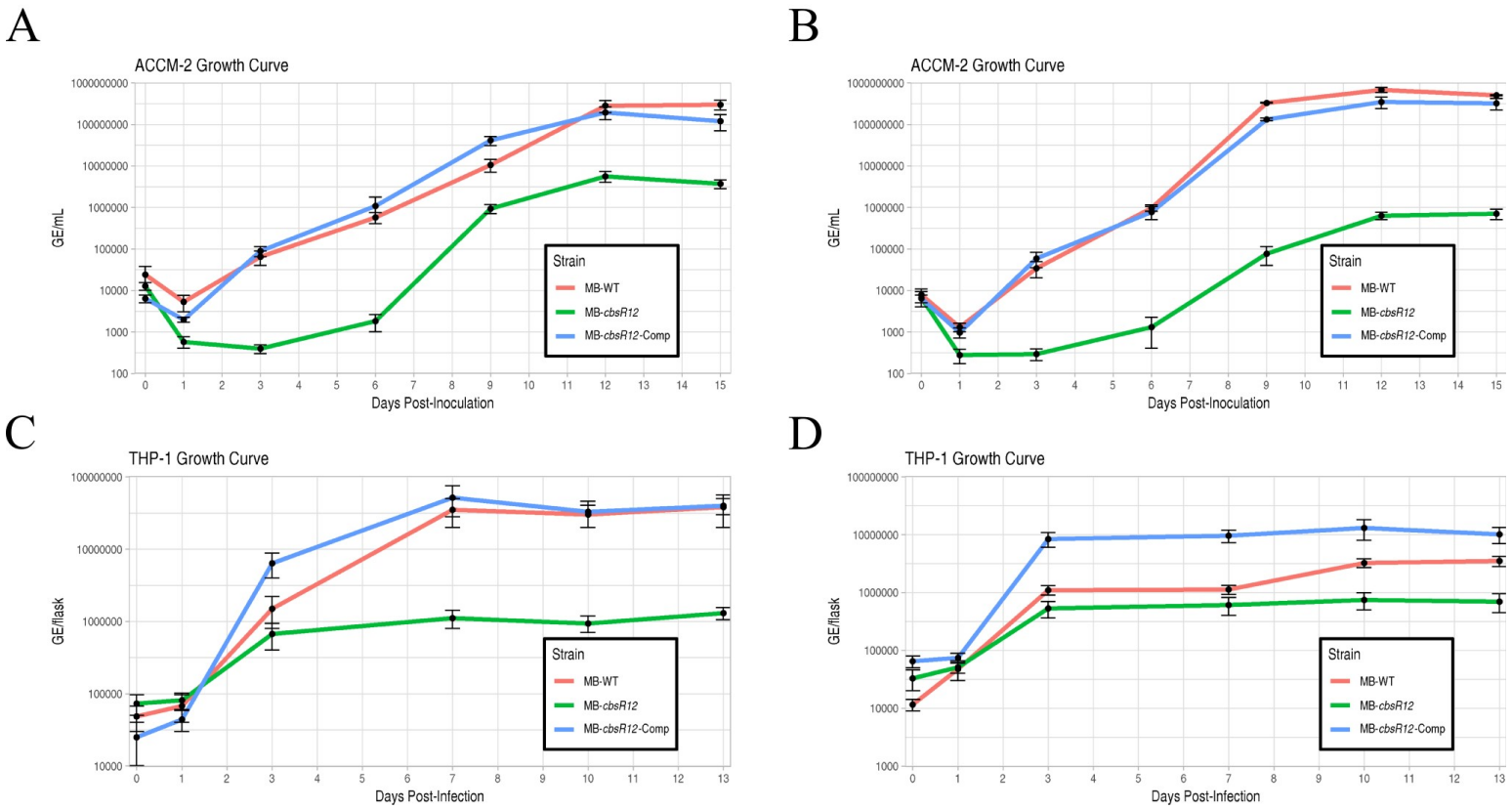
2.4
2.2
2.0
1.8
1.6
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
MB-WT
MB-cbsR12
MB-cbsR12-Comp
Strain

Fig. S3


A
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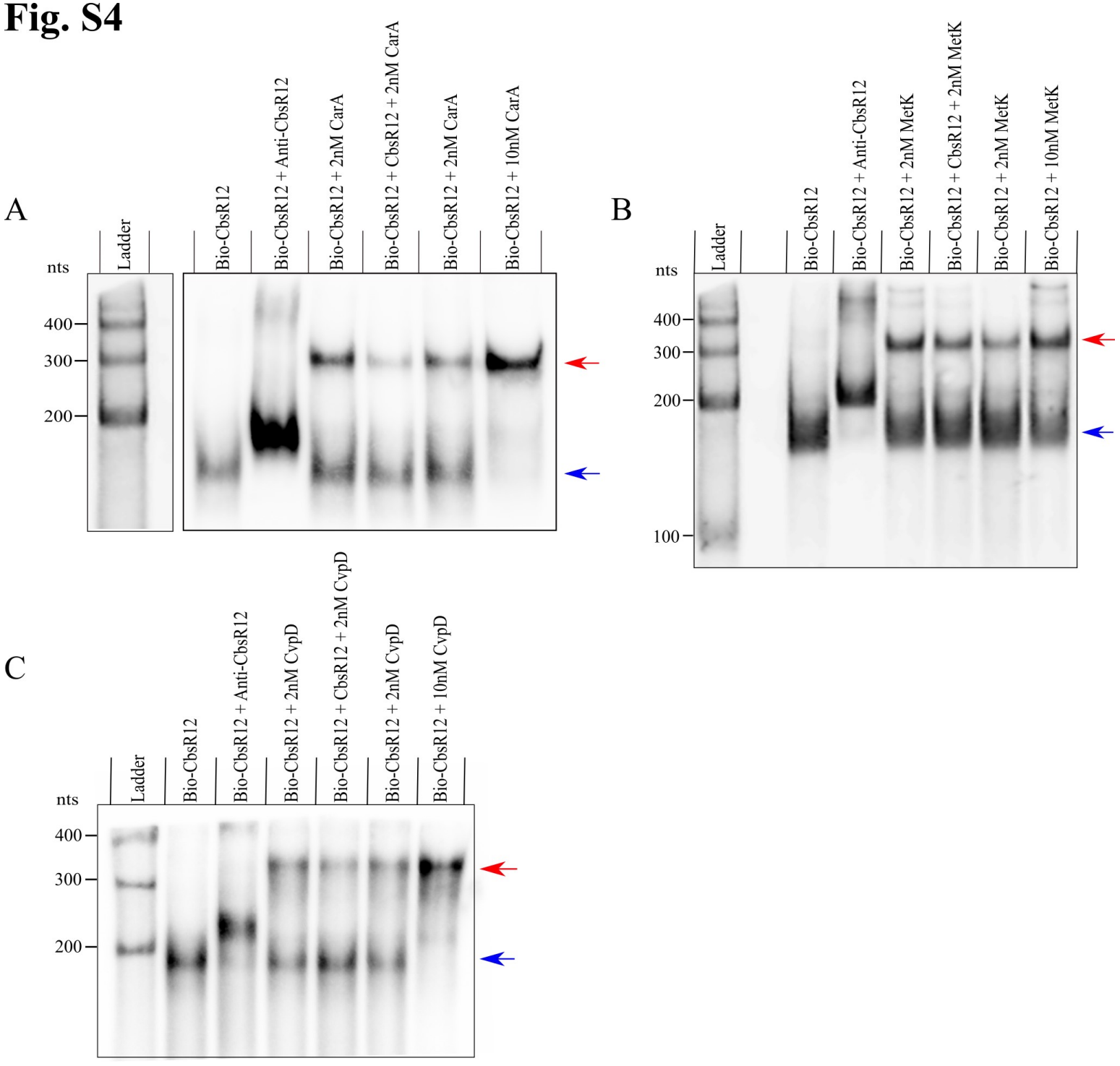
GE/mL
Days Post-Inoculation
Strain
MB-WT
MB-cbsR12
MB-cbsR12-Comp
B
ACCM-2 Growth Curve
GE/mL
Days Post-Inoculation
Strain
MB-WT
MB-cbsR12
MB-cbsR12-Comp
C
THP-1 Growth Curve
GE/flask
Days Post-Infection
Strain
MB-WT
MB-cbsR12
MB-cbsR12-Comp
D
THP-1 Growth Curve
GE/flask
Days Post-Infection
Strain
MB-WT
MB-cbsR12
MB-cbsR12-Comp

Fig. S4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nts
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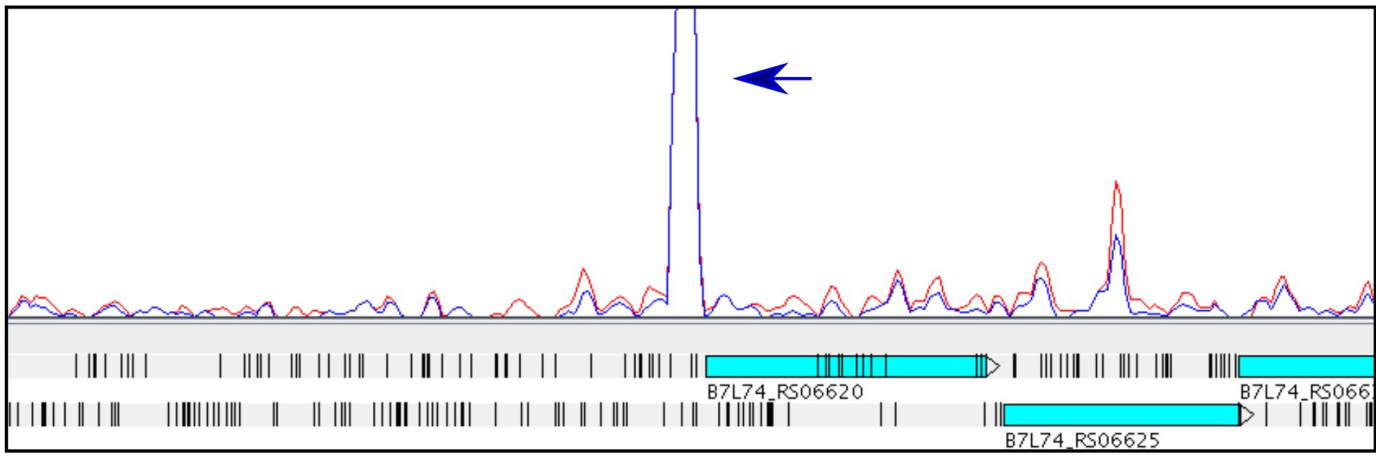
400
300
200
Bio-CbsR12
Bio-CbsR12 + Anti-CbsR12
Bio-CbsR12 + 2nM CarA
Bio-CbsR12 + CbsR12 + 2nM CarA
Bio-CbsR12 + 2nM CarA
Bio-CbsR12 + 10nM CarA
B
nts
Ladder
400
300
200
100
Bio-CbsR12
Bio-CbsR12 + Anti-CbsR12
Bio-CbsR12 + 2nM MetK
Bio-CbsR12 + CbsR12 + 2nM MetK
Bio-CbsR12 + 2nM MetK
Bio-CbsR12 + 10nM MetK
C
nts
Ladder
400
300
200
Bio-CbsR12
Bio-CbsR12 + Anti-CbsR12
Bio-CbsR12 + 2nM CvpD
Bio-CbsR12 + CbsR12 + 2nM CvpD
Bio-CbsR12 + 2nM CvpD
Bio-CbsR12 + 10nM CvpD

Fig. S5

A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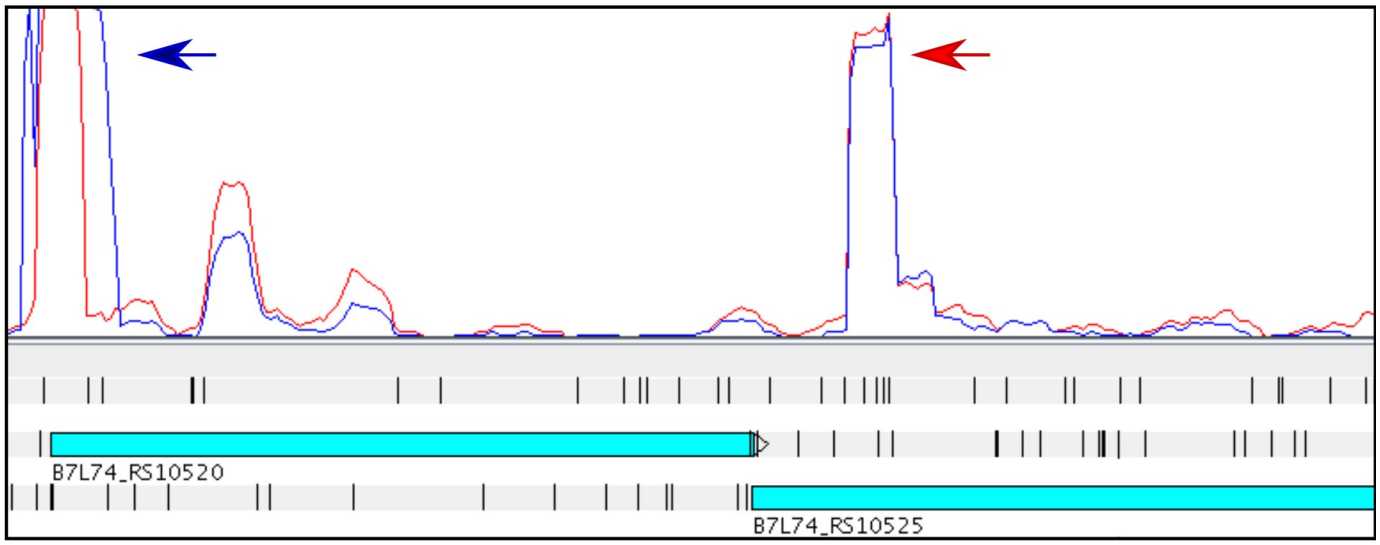
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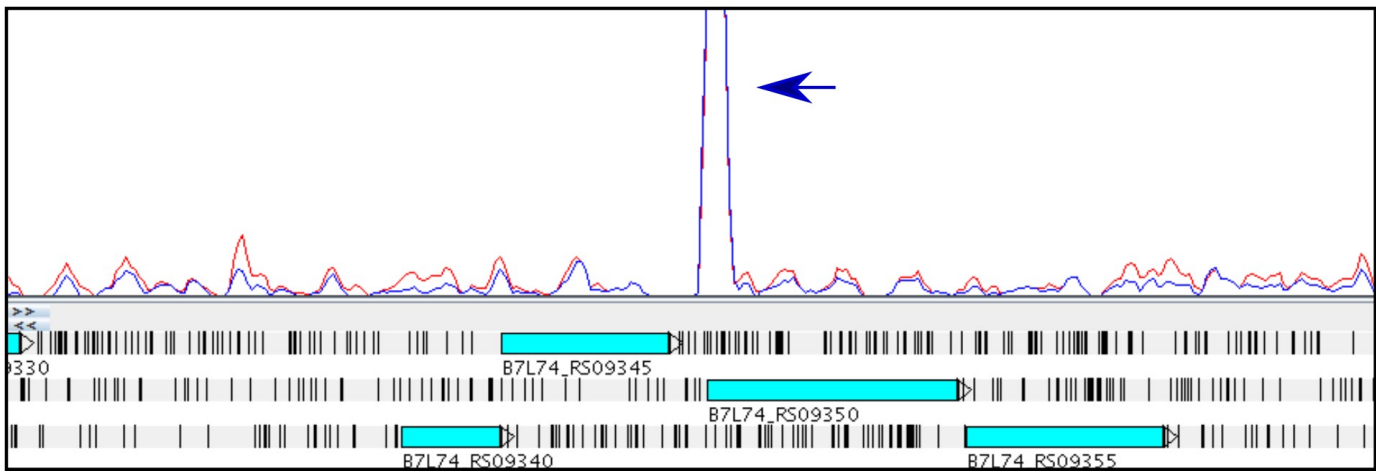
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Fig. S6

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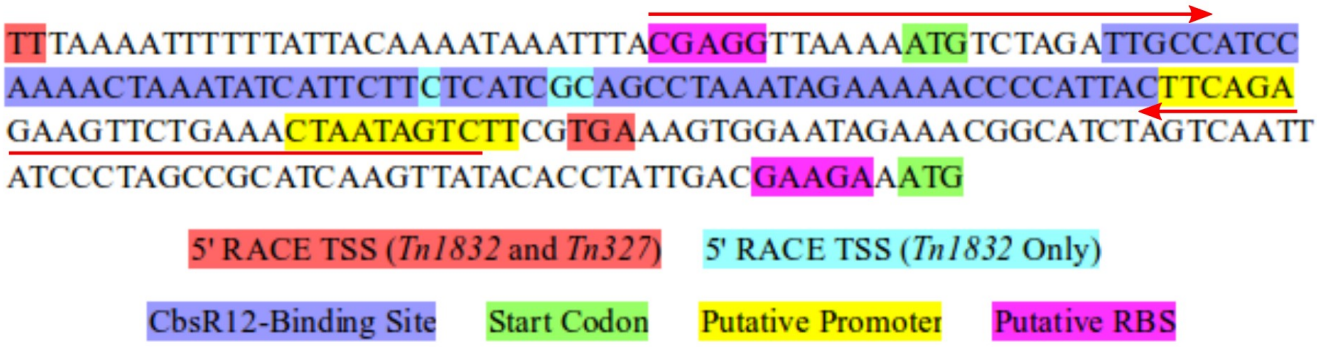
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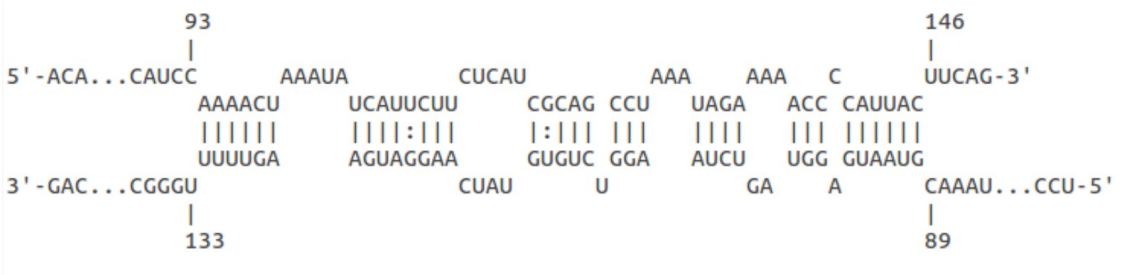
ATCCCTAGCCGCATCAAGTTATACACCTATTGACGAAGAAATG
5' RACE TSS (Tn1832 and Tn327)
5' RACE TSS (Tn1832 Only)
CbsR12-Binding Site
Start Codon
Putative Promoter
Putative RBS

B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3'-GAC...CGGGU CUAU U GA A CAAAU...CCU-5'
133
89

C

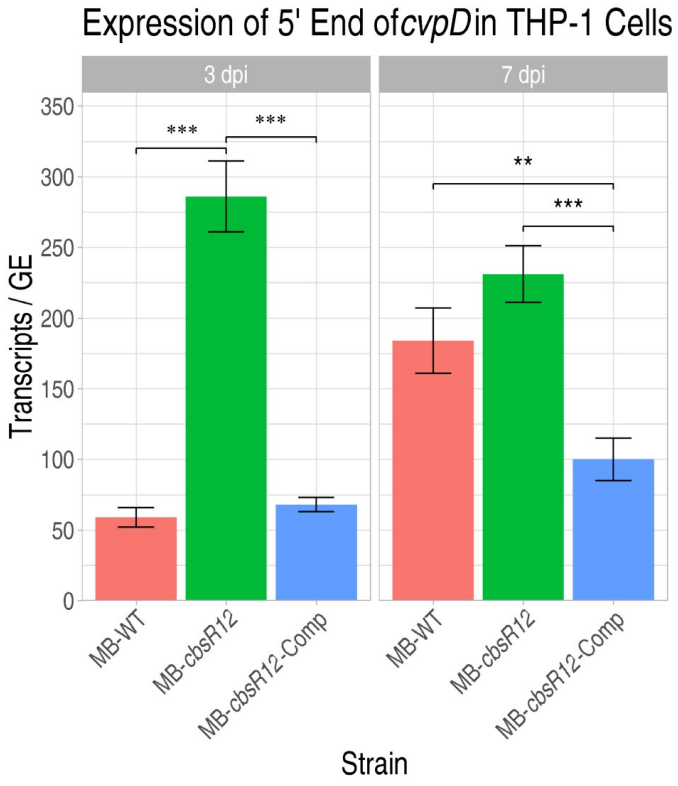
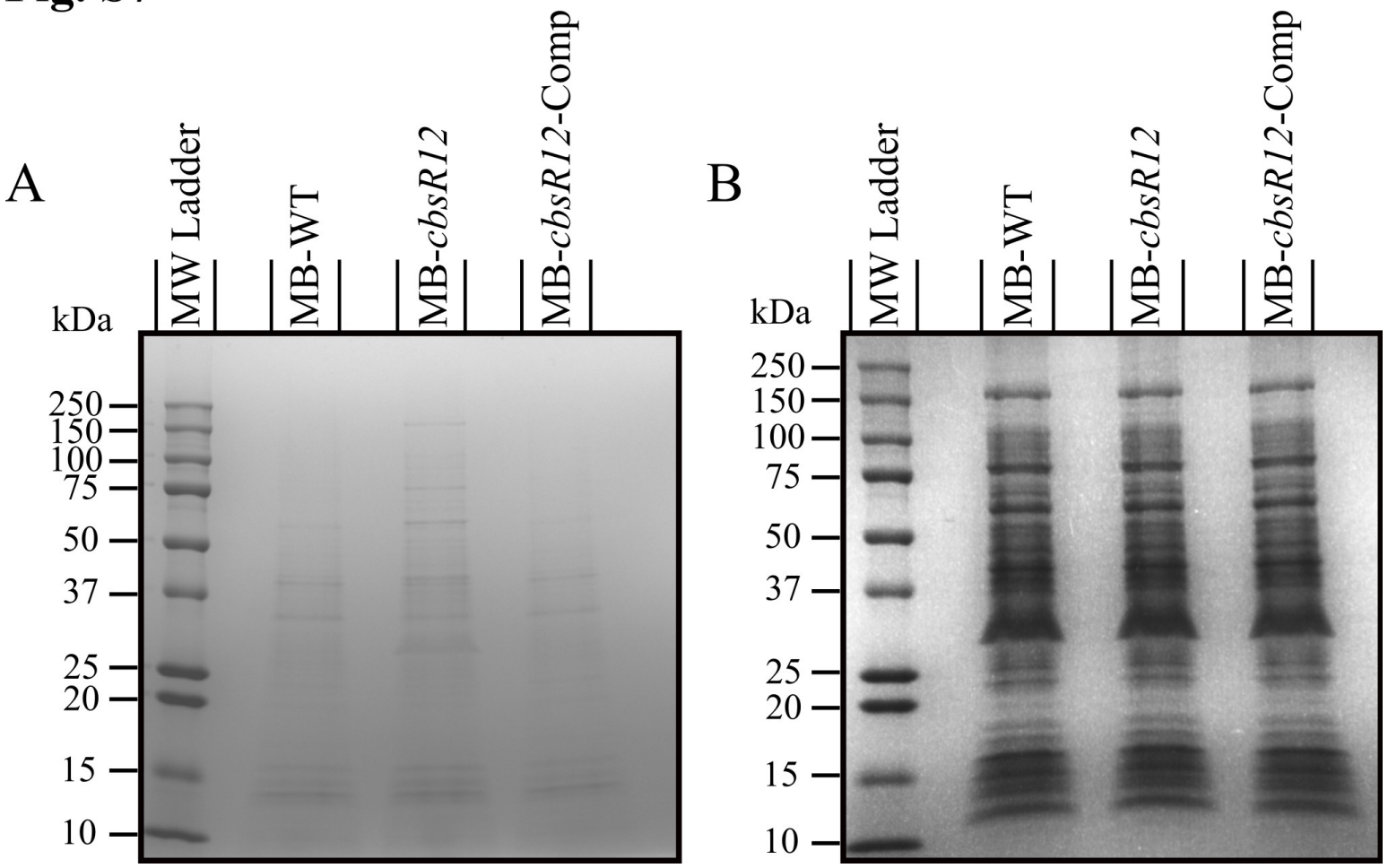

Expression of 5' End of cvpD in THP-1 Cells
3 dpi
7 dpi
Transcripts / GE
350
300
250
200
150
100
50
0
***
***
**
***
MB-WT
MB-cbsR12
MB-cbsR12-Comp
MB-WT
MB-cbsR12
MB-cbsR12-Comp
Strain

Fig. S7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MW Ladder
MB-WT
MB-cbsR12
MB-cbsR12-Comp
kDa
250
150
100
75
50
37
25
20
15
10
B
MW Ladder
MB-WT
MB-cbsR12
MB-cbsR12-Comp
kDa
250
150
100
75
50
37
25
20
15
10

Fig. S8

A


pBEST+*carA*
5'UTR
pBEST+*carA*
5'UTR +
cbsR12

B

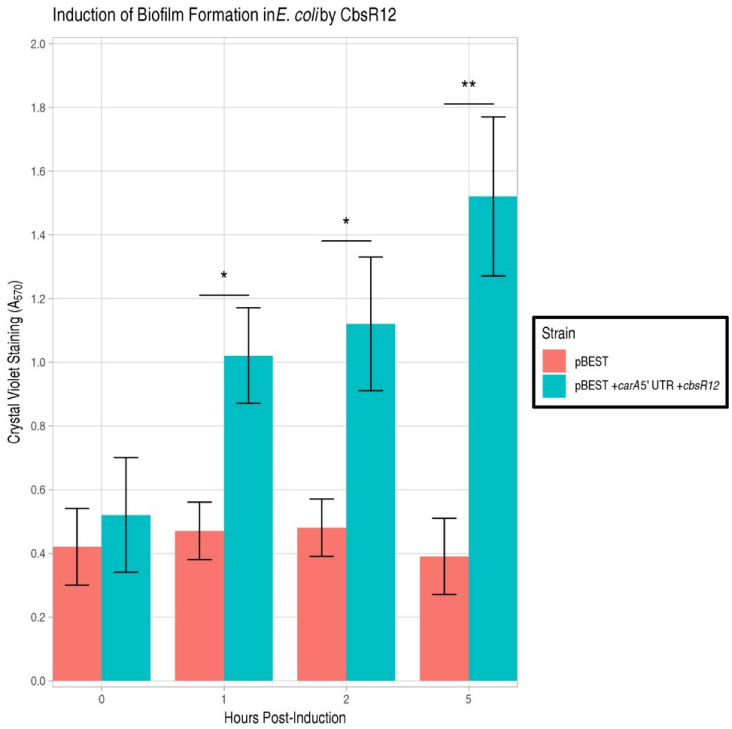
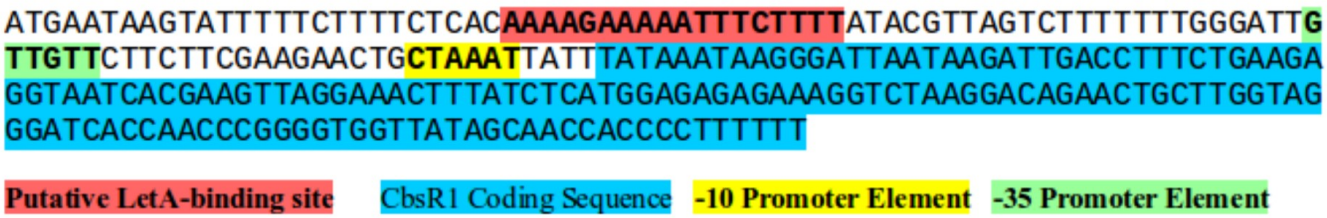

Induction of Biofilm Formation in *E. coli* by CbsR12
Crystal Violet Staining (A_{570})
2.0
1.8
1.6
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
*
*
**
0
1
2
5
Hours Post-Induction
Strain
pBEST
pBEST +*carA*5' UTR +*cbsR12*

Fig. S9

A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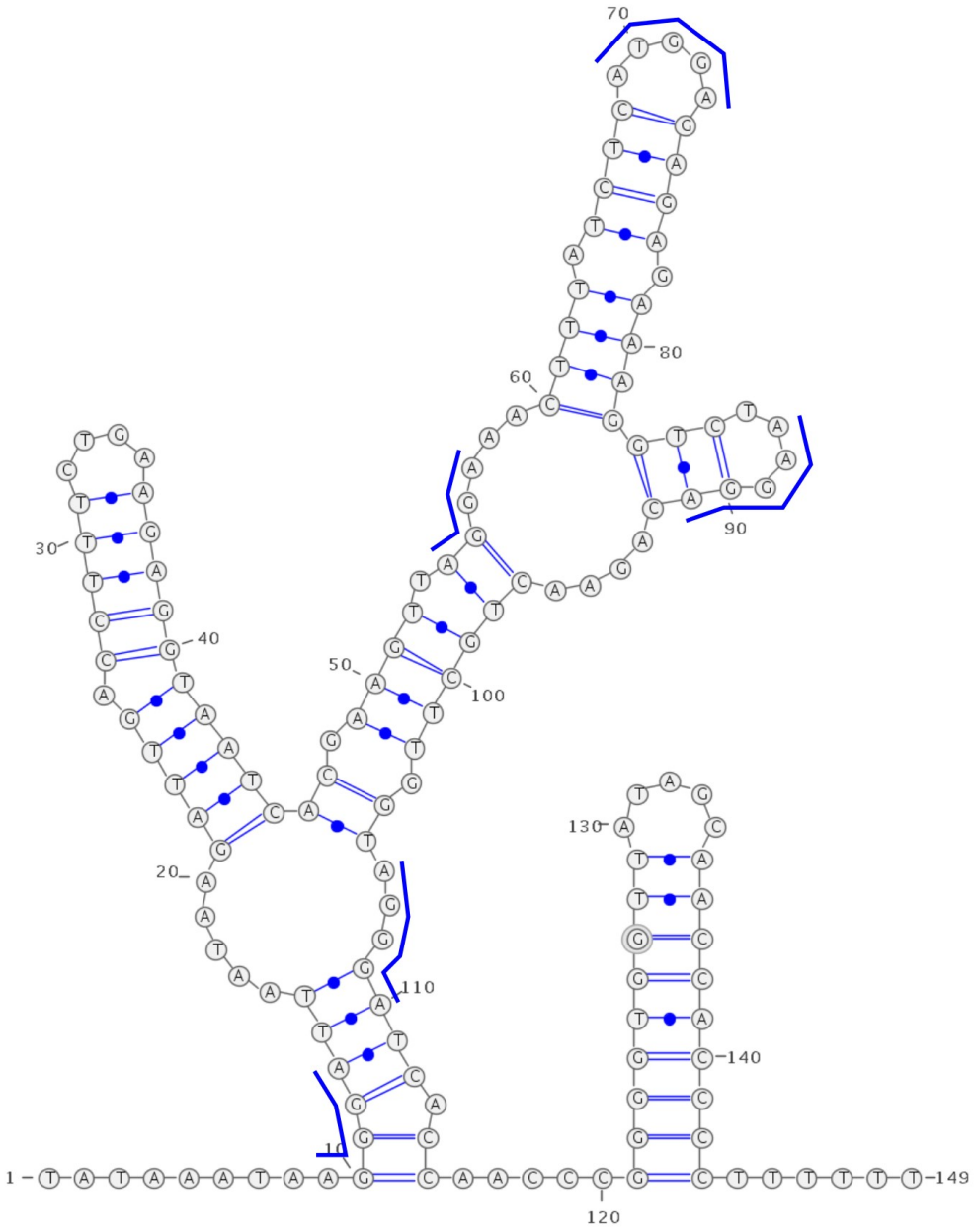
Putative LetA-binding site
CbsR1 Coding Sequence
-10 Promoter Element
-35 Promoter Element

B


1
10
20
30
40
50
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90
100
110
120
130
140
149

Fig. S10

Strains	Description	Origin
TOP10F'	E. coli Chemically Competent Strain	Invitrogen
PIR1	*E. coli* Strain with PIR Origin of Replication	Paul Beare
MB-WT	*C. burnetii* "Control" Transposon Mutant (*Tn1832*)	[2]
MB-*cbsR12*	*C. burnetii* Transposon_CbsR12 Mutant Clone (*Tn327*)	[2]
MB-*cbsR12*-Comp	Pmini-Tn7 Complement of MB-*cbsR12*	This Study

Plasmid Name	Purpose	Background	Origin
Frameshifted Luciferase	Reporter Assay Negative Control	Top 10 F'	This Study
pBEST + carA 5'UTR	Reporter Assay	Top 10 F'	This Study
pBEST + carA 5'UTR _ CbsR12	Reporter Assay	Top 10 F'	This Study
pBEST + metK	Reporter Assay	Top 10 F'	This Study
pBEST + metK + CbsR12	Reporter Assay	Top 10 F'	This Study
carA_pQE30	CarA Expression Plasmid	Top 10 F'	This Study
metK_pQE30	MetK Expression Plasmid	Top 10 F'	This Study
pQE30_rnc	RNase III Assay	Top 10 F'	[1]
csrA1_pQE30	CsrA-1 Expression Plasmid	Top 10 F'	This Study
csrA2_pQE30	CsrA-2 Expression plasmid	Top 10 F'	This Study
pMiniTnS2-ABCD	Tn327 Complementation	PIR1	[3]
pMiniTn7-CbsR12-KAN	Tn327 Complementation	PIR1	This Study
pCR2.1-TOPO	TA cloning vector	Top 10	Invitrogen

Primer Category	Primer Name	5'-3' Sequence	Reference
QRT-PCR	Q_CbsR12_F_L_qRT	GCTGATAAACAAGGTAGTTTAGCTGAGGTC	This Study
	Q_CbsR12_R_qRT	GTCTGCAGCGGGCTTCCTT	This Study
	Q_dotA_qRT_F	CTGGGAGAAGCTAAACAGGGGG	This Study
	Q_dotA_qRT_R	CCACAGCTAGCCCTGAAAAGGTATAC	This Study
	Q_cvpD_qRT_F	CGAGGTTAAAAATGTCTAGATTGCC	This Study
	Q_cvpD_qRT_R	GACTATTAGTTTCAGAACTTCTCTGAAG	This Study
EMSA	NEW_QCbsR12_F_T7	TAATACGACTCACTATAGGGGAAACGTGAGTGTTTAG	This Study
	Q_CbsR12_R_GOOD	CTCTTTATTAGTACAATCTGGAGGGTCTGCAGC	This Study
	QmetK_F+T7	TAATACGACTCACTATAGGTGAAACATTAATTTAGG	This Study
	QmetK_R	GGTCTTGGCCAATCAGGG	This Study
	QcarA_F+T7	TAATACGACTCACTATAGGCTCTAAAGTAACTCAACC	This Study
	QcarA_R	GCGGCAGCACCCTCTTTACCTA	This Study
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	QpurH_F+T7	TAATACGACTCACTATAGGAGAGTGGTTATGCGCTAC	This Study
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	QdnaA_F+T7	TAATACGACTCACTATAGGAAAACTTTAATTTCTTTTCTTTCCA	This Study
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	QrpsA_F+T7	TAATACGACTCACTATAGGGAATCGTAAACAGACCCTAACC	This Study
	QrpsA_R	GCCTTGACCAAGGCTCCAGGAC	This Study
	QCbsR12A_F+T7	TAATACGACTCACTATAGGCTGGAGGGTCTGCAGCG	This Study
	QCbsR12A_R	GCTGATAAACAAGGTAGTTTAGCTGAGGTC	This Study
Reporter Assay	LucF_RepAss_carA	AAGCTTTGTTGACAATTAATCATCGGCTCGTATAATGTGCGAAATCGAGAAAGACTCTAAAG	This Study
	LucR_RepAssATG_carA	GGATCCCGCGGCAGCACCCTCTTTACCTAT	This Study
	RA_NEWEST_CbsR12_F	GACTCGAGCTGTTGACAATTAATCATCGGCTCGTATAATGTGTGGAATTGTGAGCGGATAACAATTTCCCTCAATGGGAAACGTG	This Study
	RA_CbsR12_Rev_G3	GAAGCGCTAAGTGAGAAAAAAAAGGACCGCAAAAGCGG	This Study
	RA_LacO_Q5R	CTCACAATTCCACACATTATACGAGCCGATG	This Study
	RA_LacO_Q5F	CGGATAACAATTCGAAATCGAGAAAGACTC	This Study
	RA_NEW_MetK_Q5F	TTTACCTCAGAATCCGAAGACGCCAAAAACATAAAG	This Study
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	CbsR12_XLINK_2_F	TAATACGACTCACTATAGGGAGGACCGCAAAAGCG	This Study
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	Q_MetK_Express_F	CAGGATCCACGCACACGACCTTATTTAC	This Study
	Q_MetK_Express_R2	CACTGCAGATTGGTTACGTTTGCAGC	This Study
CsrA-1/2 Cloning	Q_CsrA1_pQE_F	CAGGATCCTTAGTCTTAACACGAACAAATG	This Study
	Q_CsrA1_pQE_R	CAAAGCTTTTCAACTTCCTCAAGAATAGGTG	This Study
	Q_CsrA2_pQE_F	CAGGATCCTTAATACTAACCAGACGTATCGG	This Study
	Q_CsrA2_pQE_R	CAAAGCTTTTCAAATTCGTCAGTCTTCTCAC	This Study
5' RACE	CbsR12_GSP2	CTTCCTTGCCCAAAAACTTCATCC	This Study
	carA_GSP1	CCAAAATCGTAAACGACC	This Study
	carA_GSP2	CGTTGAAACAGCCTTTGCTAGATCTTTTCCTTTC	This Study
	metK_GSP1	CTTTGCCGGAGAAACAC	This Study
	metK_GSP2	CGGATTGACAAGGAAACGCGTGTGTTTATC	This Study
	cvpD_GSP1	GAAGGAGAGTGAGCGG	This Study
	cvpD_GSP2	GCCTACTATTAAGCGTCTCATGATATTCAAGGGC	This Study
RNAseIII Assay	IVS_Flank_F	TAATACGACTCACTATAGGCTGGTTCTCCTCG	[1]
	IVS_Flank_R	CTTTTCCTGGAAGCGTGG	[1]
CbsR12 Complement	CbsR12_EcoRI_F	GCCCCGAATTCGGCGAAGGCTAAAGTGAGAA	This Study
	CbsR12_BamHI_R	CCTCAGGATCCGTCGGTTTTTAGCCGCTTTC	This Study
	NM2_GlmS_F	CCTATTGCATACACGATTCCACTG	This Study
	NM2_Kan_F	ATGATTGAACAGATGGATTGCACGC	This Study
	Long_CbsR12_F	TATGTTTGTTAAGGGAAGCTGAAGTG	This Study