Supporting information for Lie et al., "Overlapping repressor binding sites regulate expression of the *Methanococcus maripaludis glnK*$_1$ operon"

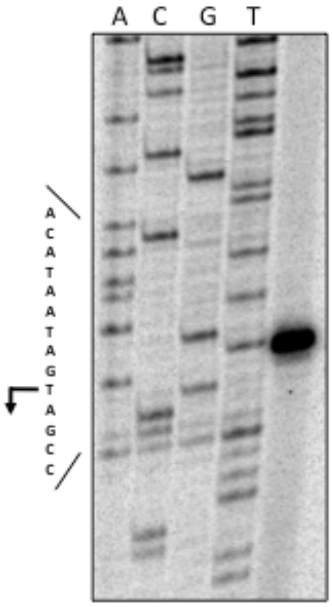

Fig. S1. Primer extension analysis of the *glnK*$_1$ mRNA

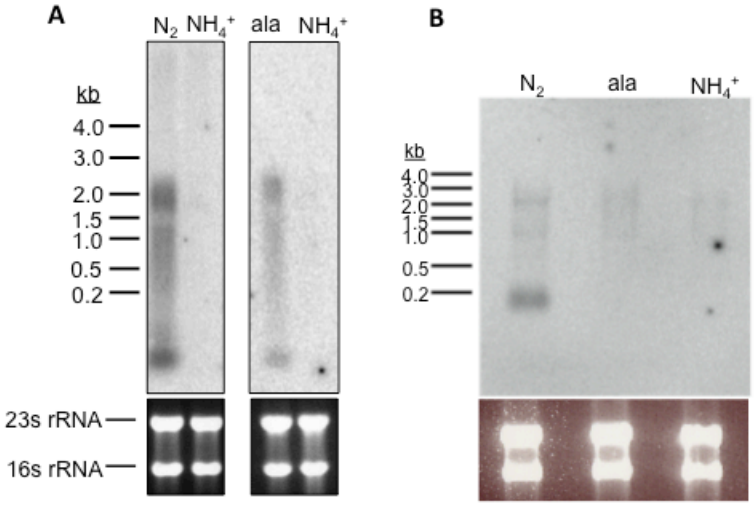

Fig. S2. Northern blots of *glnK*$_1$ mRNA

A. RNA from batch cultures of Mm900 grown with N_2 or ammonia, and alanine or ammonia, are compared in two separate hybridizations.

B. RNA was extracted from H_2-limited chemostat cultures (Haydock et al., 2004) grown on three different nitrogen sources. Ethidium bromide-stained rRNA bands show equal loading.

Sizes of RNA standards are shown.

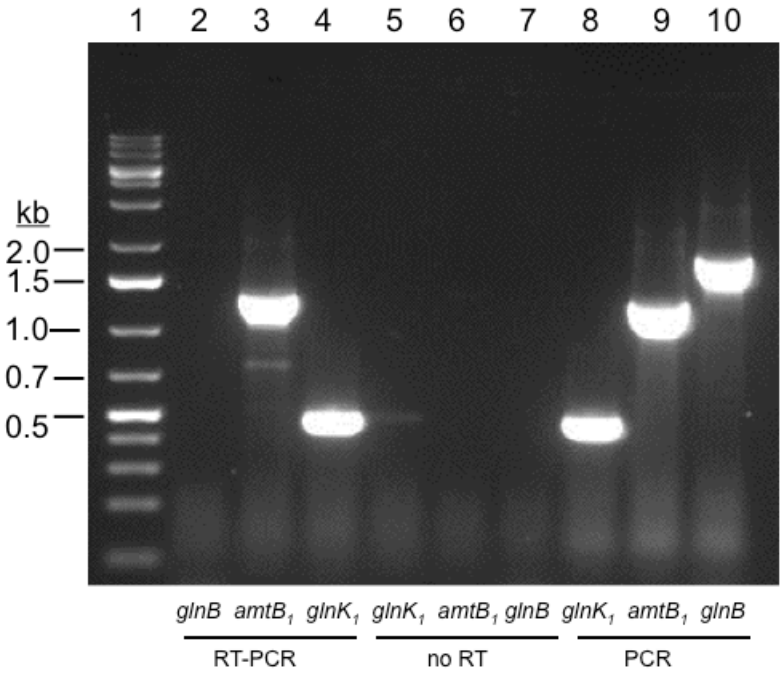

Fig. S3. Reverse transcription analysis of *glnK*$_1$ mRNA
PCR and RT-PCR reactions were performed using a forward primer in the upstream untranslated region and reverse primers in each coding region (indicated below the figure). The location of each primer is mapped in Fig. 2. RNA (1 μg per reaction) was used for RT-PCR (lanes 2-4) or PCR without RT (lanes 5-7). Genomic DNA (4 μg per reaction) was used for PCR (lanes 8-10).

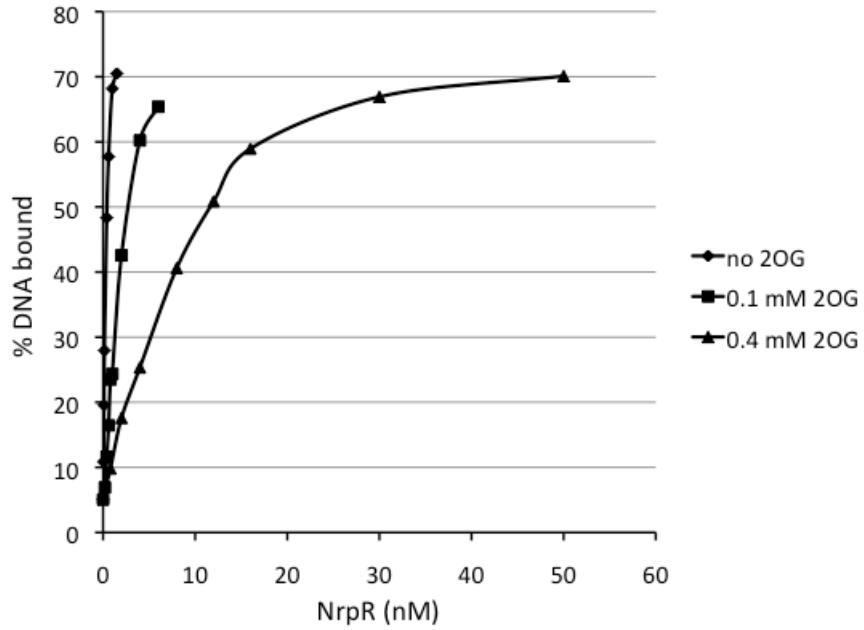

Fig. S4. NrpR binding to *glnK*$_1$ operator DNA with varying 2OG
Plots are derived from EMSA experiments. DNA probes contained *glnK*$_1$ $O_1 + O_2$ as in Fig. 3. K_d values were estimated as the NrpR concentrations at half-maximal percent shifts.

Table S1. Strains and plasmids

Strain or plasmid	Features	Reference
Strains		
S2	Wild type *M. maripaludis*	(Whitman *et al.*, 1986)
Mm900	S2 Δhpt	(Moore & Leigh, 2005)
Mm1127	Mm900 Δupt::P_{glnK1}-lacZ	This study
Mm1213	Mm900 Δupt::$P_{glnK1ct1ag1}$-lacZ[a]	This study
Mm1128	Mm900 Δupt::$P_{glnK1ct2ag2}$-lacZ[a]	This study
Mm1164	Mm900 Δupt::$P_{glnK1ct1ag1ct2ag2}$-lacZ[a]	This study
Mm1198	Mm1127 ΔnrpR	This study
Plasmids		
pCR2.1®-TOPO	Amp^r Kan^r Cloning vector	Invitrogen
pWLG40+lacZ	Amp^r Pur^r Replicative vector for *M. maripaludis*, contains P_{hmvA} of *M. voltae* fused to lacZ	(Gardner & Whitman, 1999)
pWLG40K_1	Amp^r Pur^r pWLG40 where P_{hmvA} is replaced by P_{glnK}	This study
pWLG40glnK_1O_1	Amp^r Pur^r pWLG40 where P_{hmvA} is replaced by $P_{glnKct1ag1}$[a]	This study
pWLG40glnK_1O_2	Amp^r Pur^r pWLG40 where P_{hmvA} is replaced by $P_{glnKct2ag2}$[a]	This study
pWLG40glnK_1O_1+O_2	Amp^r Pur^r pWLG40 where P_{hmvA} is replaced by $P_{glnKct1ag1ct2ag2}$[a]	This study
pBLPrt	Amp^r Kan^r Neo^r vector for markerless integration by replacing the upt gene	(Moore & Leigh, 2005)
pBLPrtglnK_1	pBLPrt with P_{glnK1}-lacZ	This study
pBLPrtglnK_1O_1	pBLPrt with $P_{glnK1ct1ag1}$-lacZ[a]	This study
pBLPrtglnK_1O_2	pBLPrt with $P_{glnK1ct2ag2}$-lacZ[a]	This study
pBLPrtglnK_1O_1+O_2	pBLPrt with $P_{glnK1ct1ag1ct2ag2}$-lacZ[a]	This study
pCRPrtNeo	vector for markerless gene replacement	(Moore & Leigh, 2005)
pCRPrtNeoΔnrp	pCRPrtNeo with in frame deletion of the nrpR gene	(Lie et al., 2007)
pMmp1.1	pGEM containing *M. maripaludis nif* promoter	(Cohen-Kupiec et al., 1997)
pCR2.1glnK_1pro	pCR2.1®-TOPO containing *M. maripaludis* $glnK_1$ promoter	This study

[a]The mutations in O_1 and O_2 are designated ct1ag1 and ct2ag2 respectively.

Table S2. Primers

Primer	Sequence (5'-3)	Restriction site
PglnK1fw	ATACTAGTGTGGGTACCATGGTTGTAAGAG	SpeI
PglnKrv	TATAATGCATACACCCCTTCGTGTCTTTG	NsiI
PglnKAscIfw	TATAGGCGCGCCGTGGGTACCATGGTTGTAAGAG	AscI
LacZAscIrv	TATAGGCGCGCCGCCCGGTTATTATTATTTTTGACAC	AscI
deltaO1rv	TCGGAAGGAACCCCTTTTCCTTAAGGGATGATAATACATTGAC	
deltaO2rv	GCGTCTCGGAACCTTCCGGAAAAGGTTTTCCGATGATAATAC	
deltaO1/O2rv	GGTGCGTCTCGGAACCTTCCCCTTAAGGTTAAGGGATGATAATA CATTG	
utrglnK1fw	AAACCAAATAACCGACATTACC	
utrglnK1rv	ACACCCCTTCGTGTCTTTG	
Exglnk1a	GGCTATTTTAAGGTTAATGG	
P7	GCTCTAGATTTGGTTGGTGCGTCTCGG	
P8	GCTCTAGATAAGTCAATGTATTATCATCGGAAAAGG	
P9	GCTCTAGATAAGTCAATGTATTATCATCCCTTAAGGAAAAGGGG	
P13	GCTCTAGATTTGGTTGGTGCGTCTCGGAAGGAACCCCTTTTCC	
P10	GCTCTAGATAAGTCAATGTATTATCATCGGAAAACCTTTTCCGG AAGGTTC	
P12	GCTCTAGATTTGGTTGGTGCGTCTCGGAACCTTCCGGAAAAGG	
P11	GCTCTAGATAAGTCAATGTATTATCATCCCTTAACCTTAAGGGG	
P14	GCTCTAGATTTGGTTGGTGCGTCTCGGAACCTTCCCCTTAAGG	
LfRepKO1	ATTATCTAGAAAAGCAATTTCGCTGAACAATGC	
RtRepKO1	ATATACTCGAGCTTTTACAACGAAATTTCC	
Mmniffor1	TTTAGTTTTTATGGGACTATTATCG	
Mmnifrev	GCATTAGGCCTCTATATATTGTTGTC	
FpglnKF	ATCTATTTATTGGTCTATTTGG	
FpglnKrev2	GGTTAATGGTGCGATATAGTGATTTGG	
FpglnKRNotI	TAAGCGGCCGCTTGGCTATTTTAAGGTTAATGG	
FpglnkFNotI	TAAGCGGCCGCATCTATTTATTGGTCTATTTGG	
Glnkutr	AAACCAAATAACCGACATTACC	
Glnk1rtr	TTATGCCTGATTCGCCTG	
Amtbrtr	GCGAGAAGTGCTCCAAT	
Glnbrtr	CCGTCTCCAGGTTTTCC	

References

Cohen-Kupiec, R., C. Blank & J. A. Leigh, (1997) Transcriptional regulation in Archaea: in vivo demonstration of a repressor binding site in a methanogen. *Proc Natl Acad Sci U S A* **94**: 1316-1320.

Gardner, W. L. & W. B. Whitman, (1999) Expression vectors for *Methanococcus maripaludis*: overexpression of acetohydroxyacid synthase and beta-galactosidase. *Genetics* **152**: 1439-1447.

Haydock, A. K., I. Porat, W. B. Whitman & J. A. Leigh, (2004) Continuous culture of *Methanococcus maripaludis* under defined nutrient conditions. *FEMS Microbiol Lett* **238**: 85-91.

Lie, T. J., J. A. Dodsworth, D. C. Nickle & J. A. Leigh, (2007) Diverse homologues of the archaeal repressor NrpR function similarly in nitrogen regulation. *FEMS Microbiol Lett* **271**: 281-288.

Moore, B. C. & J. A. Leigh, (2005) Markerless mutagenesis in *Methanococcus maripaludis* demonstrates roles for alanine dehydrogenase, alanine racemase, and alanine permease. *J Bacteriol* **187**: 972-979.

Whitman, W. B., J. Shieh, S. Sohn, D. S. Caras & U. Premachandran, (1986) Isolation and characterization of 22 mesophilic methanococci. *System. Appl. Microbiol.* **7**: 235-240.