

## Supporting Information

A role for a conserved kinase in the transcriptional control of methionine biosynthesis in *Escherichia coli* experiencing sustained nitrogen starvation

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### Materials included:

**Figure S1.** Heat maps of the  $\sigma^{38}$  regulon.

**Figure S2.** Heat maps of dysregulated genes at N- (motility-associated).

**Figure S3.** qRT-PCR complementation data for RNA seq N-24 dysregulated genes.

**Movie S1.** Phase contrast video of wild-type bacteria at N- (see attached .avi file).

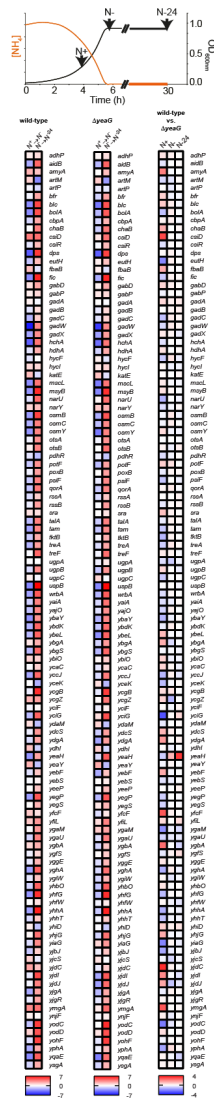
**Movie S2.** Phase contrast video of wild-type bacteria at N-24 (see attached .avi file).

**Table S1.** List of strains and plasmids used in this study.

**Table S2.** List of primers used in this study.

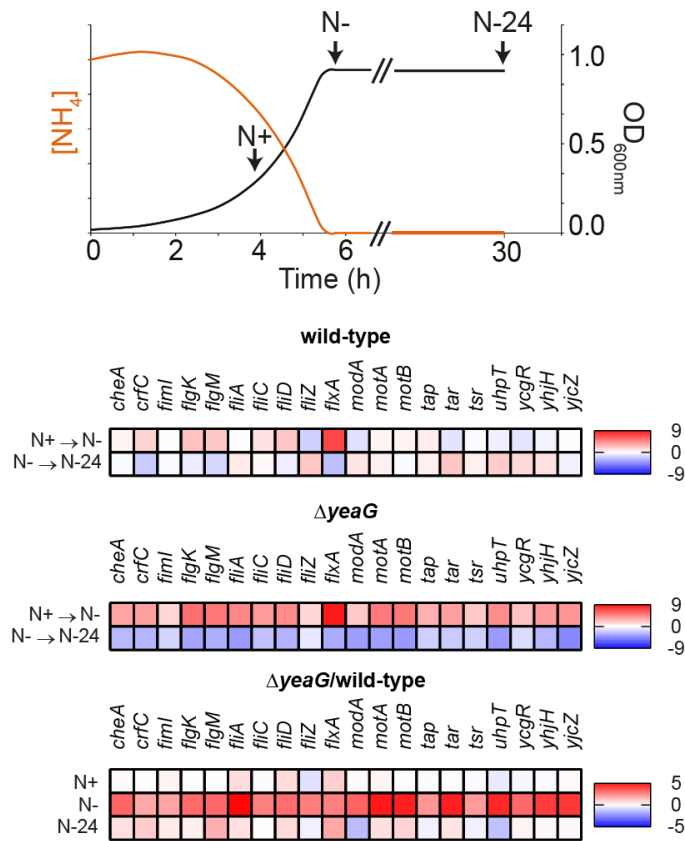
**Table S3.** Differentially expressed genes in  $\Delta yeaG$  bacteria at N- relative to wild-type bacteria.

**Table S4.** Differentially expressed genes in  $\Delta yeaG$  bacteria at N-24 relative to wild-type bacteria.



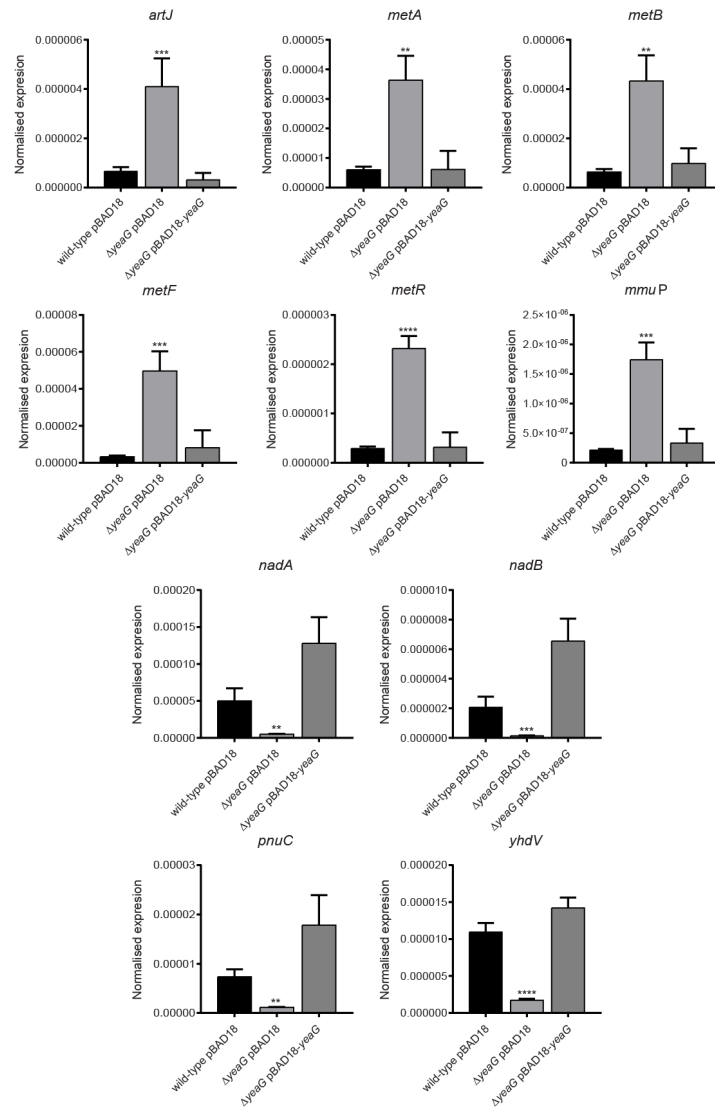
**Figure S1**

**Figure S1.** Heat map showing expression levels of the  $\sigma^{38}$  regulon (as described in (8)) in wild-type (*left*) and  $\Delta yeaG$  (*right*) bacteria at N- relative to N+, and at N-24 relative to N-, and in  $\Delta yeaG$  relative to wild-type bacteria at N+, N-, and N-24 (*right*). The colour keys at the bottom indicate the range in fold-change in relative gene expression for each comparison. The schematic representation of the bacterial growth curve at the top indicates the time points: N+, N- and N-24 with respect to the N consumption ( $[\text{NH}_4]$ ) and growth ( $\text{OD}_{600\text{nm}}$ ).



**Figure S2**

**Figure S2.** Heat map showing expression levels of motility associated genes in wild-type (*top*) and  $\Delta yeaG$  (*middle*) bacteria at N- relative to N+, and N-24 relative to N-, and in  $\Delta yeaG$  relative to wild-type bacteria at N+, N-, and N-24 (*bottom*). The colour keys on the right indicate the range in fold-change in relative gene expression for each comparison. The schematic representation of the bacterial growth curve at the top indicates the time points: N+, N- and N-24 with respect to the N consumption ( $[NH_4]$ ) and growth ( $OD_{600nm}$ ).



**Figure S3**

**Figure S3.** qRT-PCR results showing relative abundance of transcripts (normalised to 16S control expression) of *artJ*, *metA*, *metB*, *metF*, *metR*, *mmuP*, *nadA*, *nadB*, *pneC*, and *yhdV* at N-24 in wild-type (black bars),  $\Delta$ *yeaG* bacteria (light grey bars), and  $\Delta$ *yeaG* bacteria in which *yeaG* was complemented from an inducible plasmid (dark grey bars; in the presence of the inducer). See text for details. Error bars represent s.e.m. (n=3). Statistical analyses were performed by one-way ANOVA (\*\*  $p < 0.01$ , \*\*\*  $p < 0.001$ ).

## SUPPLEMENTARY MOVIES

**Movie S1.** Phase contrast video of wild-type bacteria at N-.

**Movie S2.** Phase contrast video of wild-type bacteria at N-24.

**Table S1. List of strains and plasmids used in this study**

Strains		
Name	Description	Source or Reference
Wild-type (BW25113)	<i>E. coli</i> K-12 ( <i>araD-araB</i> )567Δ( <i>rhaD-rhaB</i> )568Δ <i>lacZ</i> 4787 ( <i>::rrnB-3</i> ) <i>hsdR514 rph-1</i>	<i>E. coli</i> Genetic Stock Center
Δ <i>yeaG</i> (JW1772)	BW25113 Δ <i>yeaG</i> 780:: <i>kan</i>	<i>E. coli</i> Genetic Stock Center
Δ <i>metJ</i>	BW25113 Δ <i>metJ</i> :: <i>kan</i>	This study
<i>metJ</i> -FLAG	BW25113 <i>metJ</i> -3xFLAG	This study
Δ <i>yeaG metJ</i> -FLAG	JW1772-1 <i>metJ</i> -3xFLAG	This study
MC1061	<i>E. coli</i> K-12 F <sup>-</sup> λ <sup>-</sup> Δ( <i>ara-leu</i> )7697 [ <i>araD139</i> ]B/r Δ( <i>codB-lacI</i> )3 <i>galK16 galE15 e14<sup>-</sup> mcrA0 relA1 rpsL150(Str<sup>R</sup>) spoT1 mcrB1 hsdR2(r<sup>-</sup>m<sup>+</sup>)</i>	Stratagene®
Plasmids		
Name	Description	Source or reference
pBAD18- <i>yeaG</i>	pBAD18 expressing 6xHis- <i>yeaG</i> under an arabinose-inducible promoter ( <i>araC</i> ) (AmpR )	(1)
pDOC-F	pEX100T 3XFLAG:: <i>km</i>	(2)

**Table S2. List of primers used in this study****Primers for qRT-PCR**

Name	5' to 3' nucleotide sequence
16S fwd	TGCATCTGATACTGGCAAGC
16S rvs	ACCTGAGCGTCAGTCTTCGT
<i>artJ</i> fwd	CACCCGGAAGTGAAAAGTGT
<i>artJ</i> rvs	GGCTTTGTTATCCGGACGTA
<i>metA</i> fwd	GGCCTGGTGGAGTTTAATGA
<i>metA</i> rvs	GCGAGTTTGCTTAGGAATGC
<i>metB</i> fwd	GAGCGTGGTGGATAACACCT
<i>metB</i> rvs	GTCCGGGTCTTTAGCAATCA
<i>metF</i> fwd	AACTGGTTGGCGCGAATA
<i>metF</i> rvs	CCCAGCGTATGGCAAATC
<i>metR</i> fwd	ATTATTCGCCGATGTTTCGAC
<i>metR</i> rvs	CTACGCTGCACCGGATAAAT
<i>mmuP</i> fwd	CTGGTGGTCTGGCTGGTTAT
<i>mmuP</i> rvs	GGTCCAGGTCAGCCAGTAAA
<i>nadA</i> fwd	AACCGGTGGCTGTATTTCTG
<i>nadA</i> rvs	TTCCGGACTGAGAATTTTGG
<i>nadB</i> fwd	CCATTGGCGAGGTGAGTTAT
<i>nadB</i> rvs	CTCGTGCCAGTTATGCTGAA
<i>pnuC</i> fwd	CGACAAACCAGTCAGAACGA
<i>pnuC</i> rvs	TGCAAACACCGGATTGATAA
<i>yhdV</i> fwd	ATCAGGGTTCGTCTGGTTCAC
<i>yhdV</i> rvs	ATAGAGCGATGATCCCCATC

**Primers used for rate limiting semi-quantitative PCR of the *metF* promoter**

Name	5' to 3' nucleotide sequence
<i>metF</i> _p_fwd	CCCTTCGGCTTTTCCTTCAT
<i>metF</i> _p_rvs	AAGCTCATACCTTACCTCATCAA

**Table S3. Differentially expressed genes in  $\Delta yeaG$  bacteria at N- relative to wild-type bacteria.**

<b>Gene</b>	<b>Log<sub>2</sub> Fold Change</b>	<b>Adjusted <i>p</i> value</b>
<i>fliA</i>	4.85	5.54E-18
<i>motA</i>	4.50	1.39E-14
<i>motB</i>	4.37	8.22E-15
<i>tar</i>	4.35	4.04E-09
<i>uhpT</i>	4.23	1.16E-15
<i>yjcZ</i>	3.89	3.73E-14
<i>yhjH</i>	3.81	6.97E-06
<i>modA</i>	3.02	4.04E-09
<i>cheA</i>	2.96	5.84E-08
<i>flgM</i>	2.95	3.98E-06
<i>ycgR</i>	2.93	2.11E-05
<i>flgK</i>	2.91	1.83E-05
<i>fliD</i>	2.86	4.25E-06
<i>fliZ</i>	2.52	0.000394429
<i>fliC</i>	2.49	9.20E-06
<i>flxA</i>	2.49	0.000148505
<i>tap</i>	2.07	0.00500253
<i>tsr</i>	1.95	0.005997069
<i>fimI</i>	1.76	0.014091924
<i>crfC</i>	1.74	0.0418916

**Table S4. Differentially expressed genes in  $\Delta$ yeaG bacteria at N-24 relative to wild-type bacteria.**

<b>Gene</b>	<b>Log<sub>2</sub> Fold Change</b>	<b>Adjusted p value</b>	<b>Gene</b>	<b>Log<sub>2</sub> Fold Change</b>	<b>Adjusted p value</b>
<i>metF</i>	6.35	2.03E-13	<i>zapC</i>	2.29	0.007721396
<i>metA</i>	5.71	8.91E-14	<i>marR</i>	2.28	0.018989235
<i>metB</i>	5.29	5.16E-12	<i>argH</i>	2.25	0.003321725
<i>metR</i>	4.44	3.78E-07	<i>ygbA</i>	2.25	0.000904865
<i>metL</i>	4.19	0.000441159	<i>psd</i>	2.24	0.024679973
<i>ysaB</i>	3.79	0.001518954	<i>yqhC</i>	2.22	0.002368144
<i>mmuP</i>	3.72	4.21E-08	<i>trxC</i>	2.20	0.029712355
<i>artJ</i>	3.47	1.55E-07	<i>argF</i>	2.20	0.034888857
<i>yjdC</i>	3.29	0.00049423	<i>ybdH</i>	2.19	0.000101966
<i>ydeS</i>	3.22	0.031444707	<i>nemR</i>	2.17	2.16E-05
<i>metK</i>	3.08	0.002368144	<i>dacB</i>	2.17	0.027517357
<i>ybdL</i>	3.07	1.00E-07	<i>gspE</i>	2.15	0.015349722
<i>mmuM</i>	3.06	0.000760383	<i>ydjM</i>	2.12	0.005251082
<i>metJ</i>	2.98	0.017914666	<i>dacC</i>	2.08	0.036443447
<i>yfhL</i>	2.97	0.031465369	<i>cusB</i>	2.06	0.034742366
<i>recN</i>	2.97	1.42E-05	<i>gspC</i>	2.06	0.015215919
<i>yiiX</i>	2.93	0.000101966	<i>mlrA</i>	2.02	0.044274961
<i>mazE</i>	2.91	2.32E-05	<i>bdcA</i>	1.99	0.004574303
<i>hyi</i>	2.82	0.016182985	<i>nemA</i>	1.99	0.034888857
<i>lexA</i>	2.75	0.002368144	<i>hemH</i>	1.99	0.000904865
<i>ydbL</i>	2.74	0.00436513	<i>amyA</i>	1.98	0.033724337
<i>yihM</i>	2.72	0.044274961	<i>argB</i>	1.97	0.005184111
<i>gspD</i>	2.70	3.02E-05	<i>glnK</i>	1.97	0.028108774
<i>hiuH</i>	2.69	5.10E-07	<i>mazF</i>	1.96	0.000566992
<i>argC</i>	2.68	6.21E-06	<i>metC</i>	1.96	0.006524094
<i>yghS</i>	2.67	0.044274961	<i>recA</i>	1.95	0.010041104
<i>yjjJ</i>	2.67	0.000251888	<i>ybjI</i>	1.94	0.033650828
<i>era</i>	2.66	0.005018997	<i>glnH</i>	1.93	0.035054287
<i>ymgA</i>	2.62	0.037371218	<i>carB</i>	1.92	0.044274961
<i>yfcF</i>	2.62	0.001201977	<i>yfcD</i>	1.91	0.004854514
<i>casB</i>	2.60	0.005251082	<i>opgB</i>	1.90	0.044274961
<i>carA</i>	2.58	0.001210561	<i>dinB</i>	1.86	0.004319575
<i>fliY</i>	2.57	0.036443447	<i>hmp</i>	1.83	0.000316995
<i>argA</i>	2.55	0.006524094	<i>ypjA</i>	1.83	0.002475129
<i>yhbS</i>	2.51	0.000856934	<i>yjfD</i>	1.81	0.033164239
<i>argG</i>	2.50	0.000221252	<i>ddpX</i>	1.80	0.004574303
<i>pspA</i>	2.43	0.001541847	<i>queA</i>	1.79	0.049247204
<i>ycfP</i>	2.42	0.038564968	<i>casC</i>	1.76	0.044917048
<i>yccT</i>	2.41	2.66E-06	<i>rmuC</i>	1.75	0.002866567
<i>gloA</i>	2.40	0.000904865	<i>guaD</i>	1.73	0.008884935
<i>metN</i>	2.37	0.005388586	<i>ibpA</i>	1.71	0.002910635
<i>hyaB</i>	2.34	0.017625157	<i>lolC</i>	1.70	0.008955575



Gene	Log <sub>2</sub> Fold Change	Adjusted <i>p</i> value	Gene	Log <sub>2</sub> Fold Change	Adjusted <i>p</i> value
<i>ygdG</i>	1.70	0.03493436	<i>sseB</i>	-1.34	0.036863778
<i>yegH</i>	1.69	0.004739586	<i>cbdB</i>	-1.38	0.010041104
<i>ygiM</i>	1.69	0.017625157	<i>ydfJ_1</i>	-1.39	0.017199998
<i>arnB</i>	1.69	0.016891107	<i>aslA</i>	-1.41	0.038879805
<i>hipA</i>	1.67	0.008351201	<i>yidC</i>	-1.42	0.043508234
<i>manX</i>	1.67	0.009421219	<i>yobA</i>	-1.43	0.036863778
<i>argD</i>	1.67	0.022296223	<i>prfC</i>	-1.44	0.036443447
<i>arnC</i>	1.64	0.006762404	<i>speC</i>	-1.45	0.009865763
<i>srmB</i>	1.64	0.009959883	<i>lepB</i>	-1.45	0.00355906
<i>nanM</i>	1.62	0.004574303	<i>yffL</i>	-1.47	0.044274961
<i>yedK</i>	1.60	0.031465369	<i>rsmB</i>	-1.48	0.012827374
<i>hns</i>	1.59	0.035334862	<i>trkA</i>	-1.48	0.046253495
<i>acpH</i>	1.57	0.004574303	<i>purR</i>	-1.48	0.044274961
<i>rimO</i>	1.52	0.002426303	<i>dmsD</i>	-1.48	0.024354171
<i>pfkB</i>	1.52	0.034921441	<i>yggX</i>	-1.49	0.002341076
<i>norR</i>	1.52	0.036443447	<i>nnr</i>	-1.50	0.033881071
<i>cmoA</i>	1.51	0.044274961	<i>yfeO</i>	-1.51	0.004378689
<i>adk</i>	1.50	0.034888857	<i>yciA</i>	-1.53	0.011532221
<i>yoeG</i>	1.49	0.022994207	<i>folC</i>	-1.54	0.034888857
<i>yhiN</i>	1.47	0.024354171	<i>ykfA</i>	-1.54	0.024857104
<i>yfdE</i>	1.46	0.019770045	<i>dkgA</i>	-1.56	0.014809737
<i>glnQ</i>	1.44	0.010041104	<i>narV</i>	-1.60	0.006524094
<i>dbpA</i>	1.43	0.007721396	<i>ycgL</i>	-1.60	0.01166184
<i>rnt</i>	1.43	0.036443447	<i>yohD</i>	-1.62	0.004361867
<i>ykgF</i>	1.41	0.024354171	<i>satP</i>	-1.62	0.011904371
<i>idi</i>	1.38	0.018102718	<i>rcnB</i>	-1.63	0.004186425
<i>hicB</i>	1.37	0.009421219	<i>yhfA</i>	-1.63	0.001932853
<i>cueO</i>	1.36	0.036443447	<i>fabF</i>	-1.65	0.036443447
<i>yebF</i>	1.31	0.036863778	<i>ybcJ</i>	-1.69	0.036443447
<i>yoaD</i>	1.29	0.046253495	<i>yjcB</i>	-1.70	0.003158468
<i>yfeY</i>	1.23	0.043508234	<i>rbn</i>	-1.71	0.013126201
<i>yafN</i>	1.18	0.034888857	<i>yqjG</i>	-1.81	0.003538829
<i>ygaP</i>	1.18	0.036443447	<i>parC</i>	-1.81	0.017176033
<i>yqjF</i>	1.14	0.029026226	<i>cspD</i>	-1.81	0.002938926
<i>prlF</i>	1.10	0.046383621	<i>yafL</i>	-1.82	0.024480757
<i>livF</i>	-1.14	0.044274961	<i>xanP</i>	-1.84	0.031465369
<i>etk</i>	-1.17	0.033881071	<i>leuB</i>	-1.84	0.000279367
<i>narU</i>	-1.18	0.044274961	<i>xylA</i>	-1.87	0.036443447
<i>slyX</i>	-1.18	0.041855646	<i>eutH</i>	-1.88	0.001642406
<i>rlmJ</i>	-1.22	0.049247204	<i>tsaD</i>	-1.90	0.036443447
<i>phnP</i>	-1.23	0.04792992	<i>kdgK</i>	-1.93	0.044274961
<i>mraY</i>	-1.24	0.036443447	<i>leuA</i>	-1.94	0.000251888
<i>ybdZ</i>	-1.31	0.011498403	<i>curA</i>	-1.96	3.62E-05
<i>modA</i>	-1.34	0.027517357	<i>yecE</i>	-2.01	0.027517357

<b>Gene</b>	<b>Log<sub>2</sub> Fold Change</b>	<b>Adjusted <i>p</i> value</b>
<i>leuD</i>	-2.05	0.007422015
<i>ydcA</i>	-2.14	0.003618422
<i>yaiC</i>	-2.16	0.013126201
<i>ypfG</i>	-2.22	0.013493579
<i>ldrB</i>	-2.23	0.007046702
<i>rhtB</i>	-2.24	4.40E-05
<i>prpD</i>	-2.27	0.024679973
<i>cysZ</i>	-2.31	0.014236228
<i>leuC</i>	-2.34	2.78E-06
<i>menA</i>	-2.37	1.51E-05
<i>coaA</i>	-2.38	0.029683077
<i>yfaZ</i>	-2.41	0.006939919
<i>ybjO</i>	-2.41	0.004474186
<i>lgoD</i>	-2.43	0.046253495
<i>pheA</i>	-2.56	8.62E-06
<i>ycgM</i>	-2.70	0.00308444
<i>yciG</i>	-2.71	0.009421219
<i>allB</i>	-2.89	0.005737942
<i>ldrA</i>	-2.94	0.000207817
<i>add</i>	-2.99	0.000108515
<i>ygeG</i>	-3.38	0.046383621
<i>ldrC</i>	-3.41	2.92E-06
<i>yhdV</i>	-3.46	1.44E-09
<i>pnuC</i>	-3.52	2.55E-08
<i>nadA</i>	-3.81	1.28E-10
<i>nadB</i>	-4.64	1.54E-19
<i>ymdF</i>	-6.27	5.10E-13

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2. Lee, D. J., Bingle, L. E., Heurlier, K., Pallen, M. J., Penn, C. W., Busby, S. J., & Hobman, J. L. (2009). Gene doctoring: A method for recombineering in laboratory and pathogenic *Escherichia coli* strains. *BMC Microbiology*, 9, 1–14.