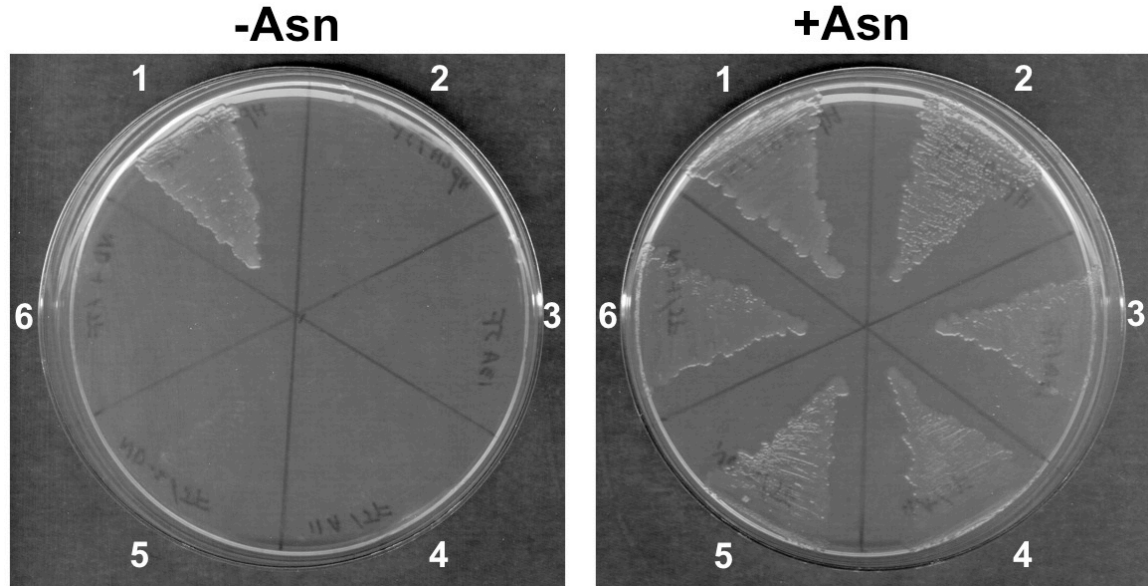


## Supplemental Figures

			▼					
<i>A. aeolicus</i>	120	FAMGSSTEYS	AFFPTKNPWD	LERVPGGSSG	GSAASVAVLS			
<i>B. subtilis</i>	127	FAMGSSTENS	AYKLTKNPWN	LDTVPGGSSG	GSAAAVAAGE			
<i>H. pylori</i>	101	FAMGSTTESS	CYGITKNPRD	KNIVPGGSSG	GSAAAVAGGL			
<i>N. meningitidis</i>	124	FAMGSTNENS	FYGAAKNPWN	LEHVPGGSSG	GSAAVVAARL			
<i>S. aureus</i>	127	FAMCGSTETS	YFKKTVNPFD	HKAVPGGSSG	GSAAAVAAGL			
<i>M. thermautotrophicus</i>	125	FAAGSSTETS	FFGPTDNPAA	PGRIPGGSSG	GSAAAVAAGM			
			▼					
<i>A. aeolicus</i>	160	APVSLGSDTG	GSIRQPASFC	GVIQIKPTYG	RVSRYGLVAF			
<i>B. subtilis</i>	167	VPFSLGSDTG	GSIRQPASFC	GVVGLKPTYG	RVSRYGLVAF			
<i>H. pylori</i>	141	TVAALGSDTG	GSIRQPASYC	GCVGLKPTYG	RVSRYGVIAY			
<i>N. meningitidis</i>	164	APAALGSDTG	GSIRQPASFC	GITIKPTYG	TVSRFGMVAY			
<i>S. aureus</i>	167	VPLSLGSDTG	GSIRQPAYYC	GVVGMKPTYG	RVSRYGLVAF			
<i>M. thermautotrophicus</i>	165	CDLALGSDTG	GSIRNPASFC	GVMGFKPTYG	AVSRQGLLDL			

**Supplemental Figure 1. Sequence alignment of the active site of GatA from bacteria (blue) and archaea (green).** GatA sequences selected from GatCAB enzymes with structural or biochemical data for amide donor preference. Residues highlighted in black are highly conserved amongst GatA polypeptides. The ▼ denotes a residue in the *A. aeolicus* GatCAB structure implicated in the enzyme being able to use both Gln and Asn as amide donors. The ▼ denotes the Ser residue in GatA that serves as the nucleophile.



**Supplemental Figure 2. Rescue of the Asn auxotrophy of *E. coli* JF448 strain by co-expression of *Deinococcus radiodurans* ND-AspRS and *Helicobacter pylori* GatCAB requires both Mg<sup>2+</sup> binding sites in B-subunit active pocket.** *E. coli* JF448 cells were transformed with a pCBS2 plasmid encoding *D. radiodurans* ND-AspRS with either 1) wild-type *H. pylori* GatCAB, 2) *H. pylori* GatCA, or 3-6) mutant *H. pylori* GatCAB enzymes [3) Glu11Ala mutation in B-subunit, 4) His13Ala mutation in B-subunit, 5) Deletion of the first 18 residues in the B-subunit, and 6) Deletion of the first 30 residues in the B-subunit]. The resulting strains were grown on M9 minimal media agar plates with (right panel) or without (left panel) Asn. *H. pylori* GatB Glu11 is equivalent to *A. aeolicus* GatB Glu12 which coordinates the Mg<sup>2+</sup> in the transient metal site. *H. pylori* GatB His13 is equivalent to *A. aeolicus* GatB His14 which coordinates the Mg<sup>2+</sup> in the permanent metal site.

			▼▼			▼▼
<i>A. aeolicus</i>	20	KTKMFCGCKV		38	NVCPVCLGMP	
<i>A. thalina</i>	150	LTKAFSCSN		168	SICPVMGLP	
<i>D. desulfuricans</i>	22	KTKLFCNCST		40	HVCPVCLGLP	
<i>G. violaceus</i>	26	ETKLFRCST		43	NICPICTGQP	
<i>H. pylori</i>	18	KTKLFCSCST		36	NTCPVCLGLP	
<i>S. avermitilis</i>	33	KT MFCGCST		51	QTCPVCLGMP	
<i>T. thermophilus</i>	17	RTKMFCGCRA		36	HTCPVCLGLP	
<i>T. pallidum</i>	41	KTKAFACAN		59	RVCPVCLGLP	
<i>A. variabilis</i>	26	NTKLFSSSST		44	NIDPVMGLP	
<i>B. subtilis</i>	18	NSKLFASPT		36	QTSVIDLQYP	
<i>C. crescentus</i>	30	KSKLFSGAAV		48	QVSLVDAAMP	
<i>C. trachomatis</i>	23	ASKLFSARN		41	NISPVCTGMP	
<i>D. geothermalis</i>	116	RSKLFSA CPA		135	YTDPLTLGLP	
<i>N. meningitidis</i>	18	KSKLFSGAST		36	HASVVECALP	
<i>N. sennetsu</i>	26	KNKLFSDAPC		44	AVTLFDAAMP	
<i>P. aeruginosa PAO1</i>	18	QSKLFSGSST		36	QASLVDTLAMP	
<i>S. aureus</i>	18	DSKMFSPSPA		36	NTNVIDLAYP	
<i>A. pernix</i>	22	GSKLFCGDS		41	NVCPVCLGLP	
<i>H. marismortui</i>	26	ETKLFRCST		45	HTCPVCLGLP	
<i>M. hungatei</i>	19	KSKMFCSCST		38	HTCPVCLGLP	
<i>M. kandleri</i>	17	RTKLFDCPT		36	NTCPVCTGMP	
<i>M. maripaludis</i>	20	NSKLFQCPT		39	NICPVCTGHP	
<i>M. mazei</i>	23	KTKMFCGCST		42	HTCPVCLGLP	
<i>M. thermautotrophicus</i>	14	RSKLFRCPT		33	NICPVCLNQP	
<i>N. equitans</i>	14	ETKLFPCPKI		32	NVCEICLQYP	
<i>S. solfataricus</i>	2	KTKLFCSCPS		21	VTCPVCLGLP	
<i>A. pernix</i>	24	REKLFSC CPA		79	HSCLVEADEE	
<i>F. acidarmanus</i>	17	N-KLFCSCST		69	NSCLVEKDEE	
<i>H. marismortui</i>	24	ATKLFDCPT		80	TTCLVEEDEE	
<i>M. hungatei</i>	23	KEKLFRCPT		79	TTCLVENDEE	
<i>M. kandleri</i>	26	SRKLFRCPT		83	FSCLVELDEE	
<i>M. maripaludis</i>	23	KRKLFNCPT		79	TTCLVELDEE	
<i>M. mazei</i>	24	KEKLFRCPT		80	TTCLVENDEE	
<i>M. thermautotrophicus</i>	21	ESKLFPCRT		77	ETCLVEADEE	
<i>N. equitans</i>	14	K-KLFCES		67	NSCLVELDEE	
<i>P. aerophilum</i>	21	QRKLFCHCPP		77	TTCLVELDEE	
<i>S. solfataricus</i>	24	PHKLFNCST		79	TSCLVEADEE	
<i>P. abyssi</i>	28	K-KLFSVPS		82	LTDLVYMDEE	

**Supplemental Figure 3. Sequence alignment of the Zn<sup>2+</sup> motif of GatB from representative bacteria (blue) and archaea (green), and representative GatE sequences (maroon).** Residues highlighted in black are highly conserved in the GatB/E family. Residues highlighted in grey are highly conserved in GatE sequences. The ▼ denotes the residues that coordinate the Zn<sup>2+</sup> in the *A. aeolicus* GatCAB structure.