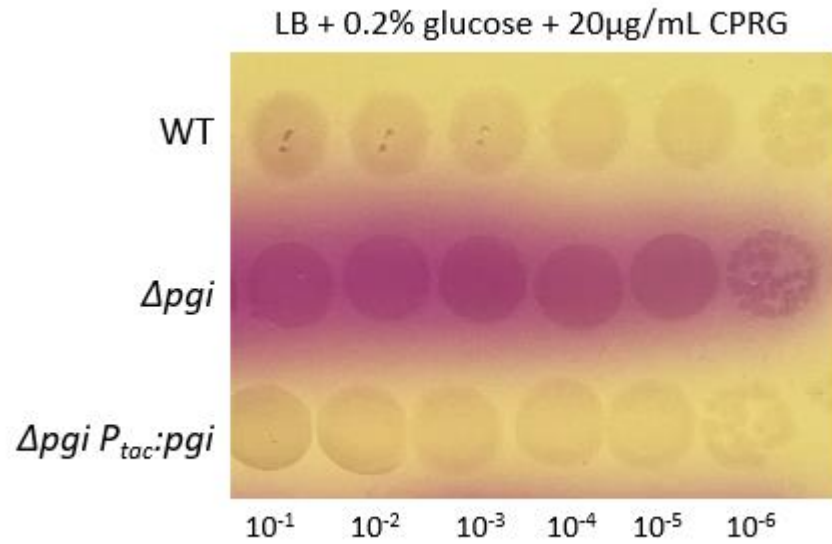


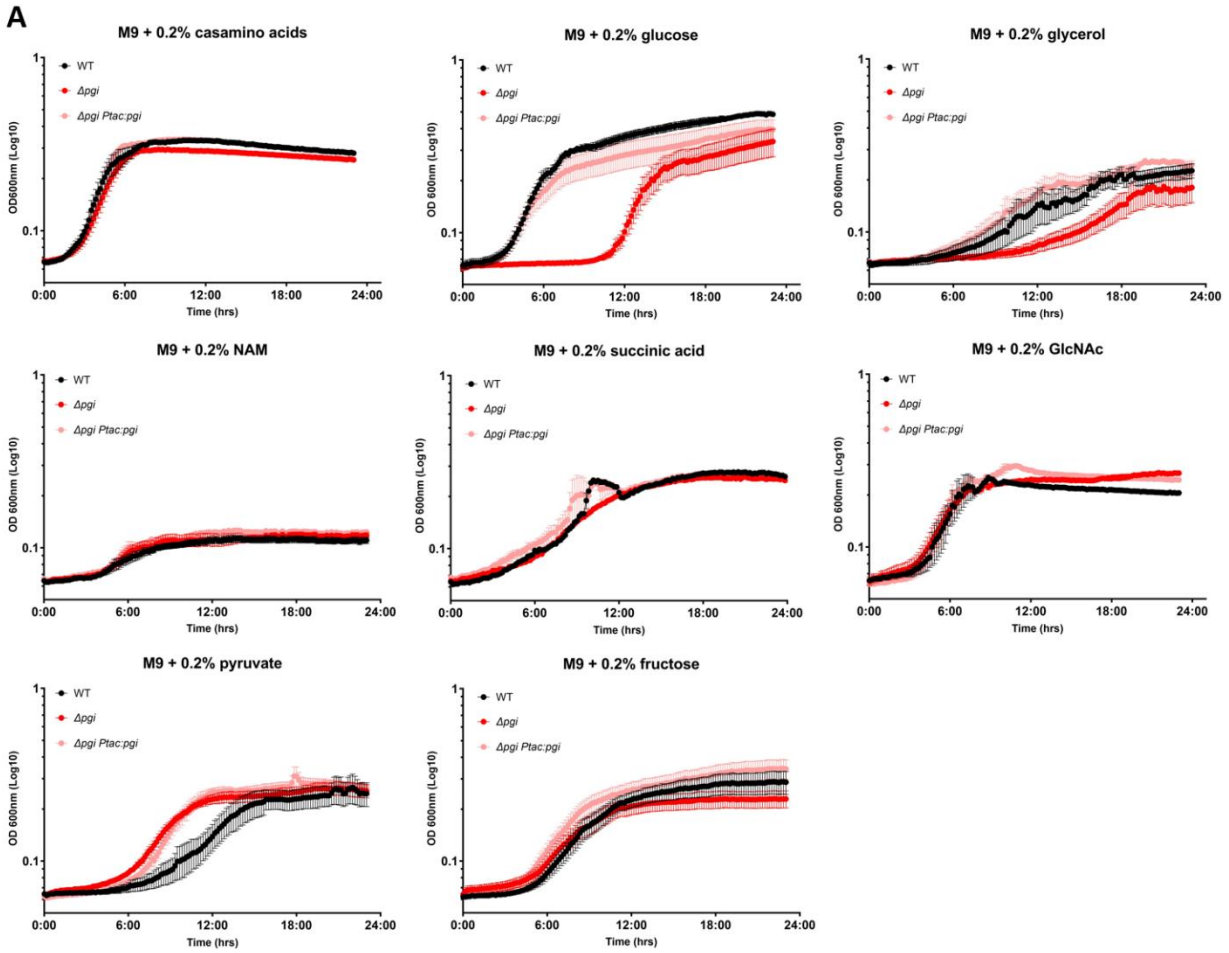
770 **Supporting Information**



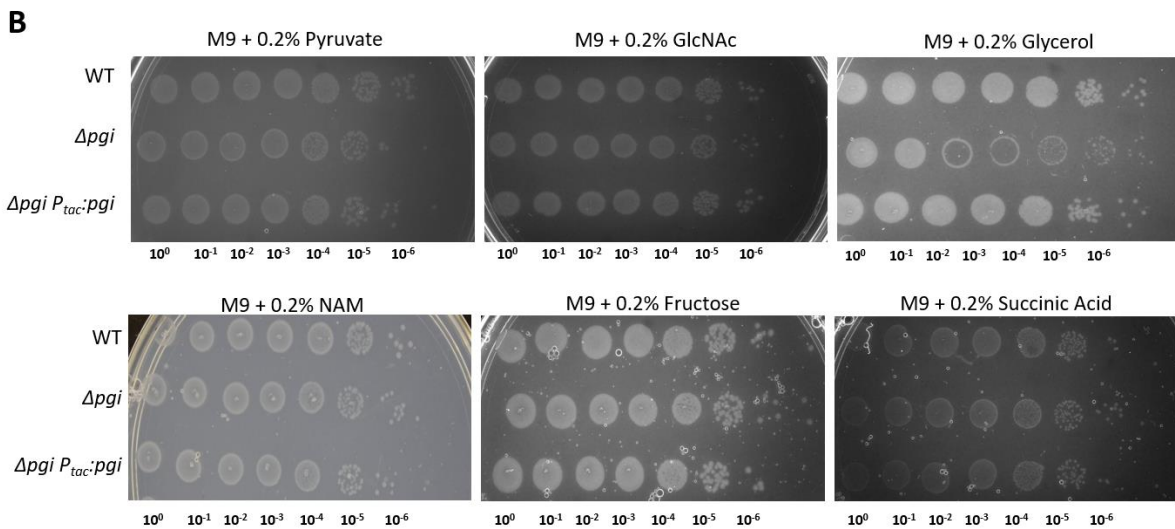
771

772 Figure S1: Enhanced lysis of a *pgi* mutant. Overnight cultures of the indicated strains  
773 were plated on agar containing glucose and the lysis indicator CPRG (see text for details)  
774 and imaged after 18 hours of growth.

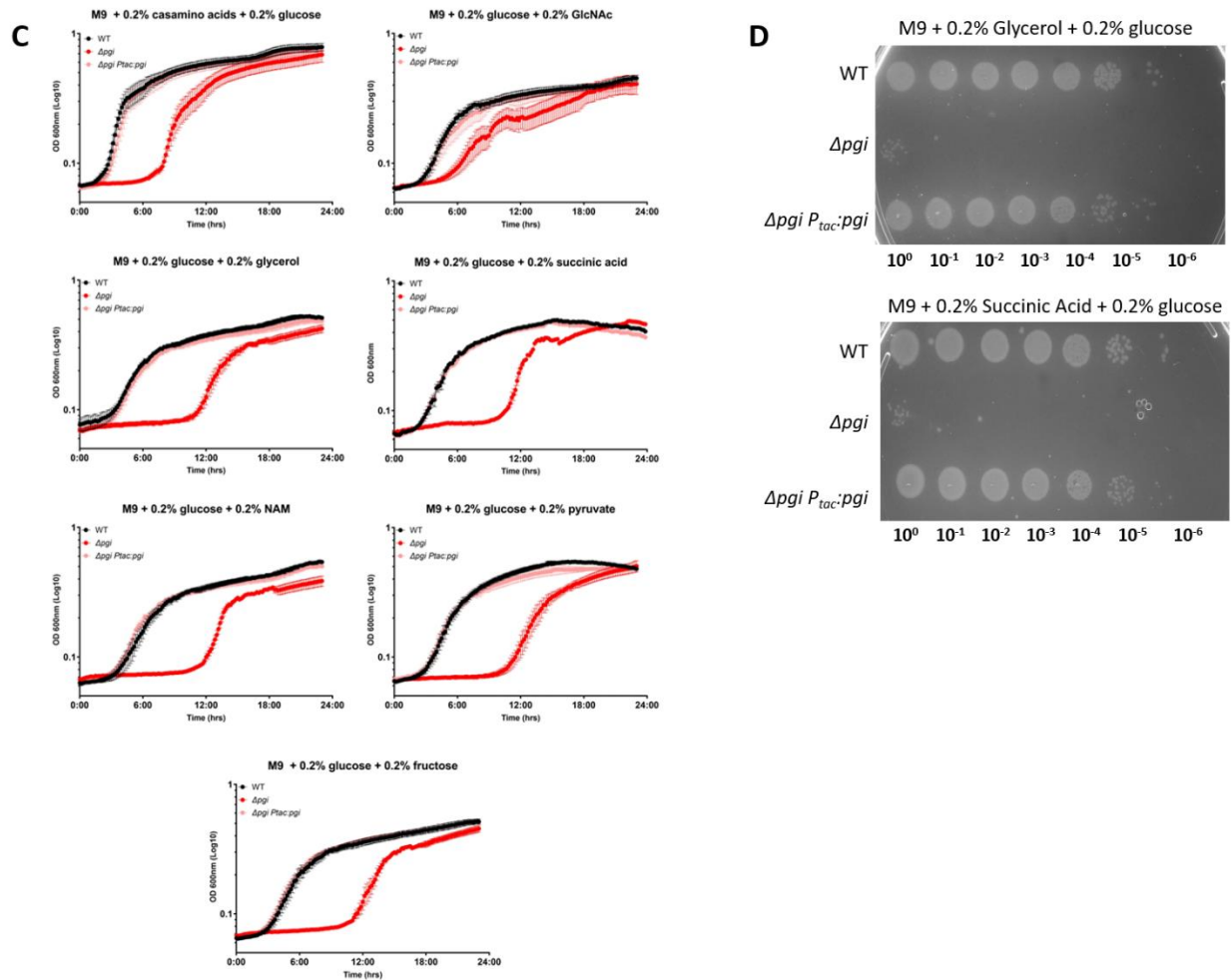
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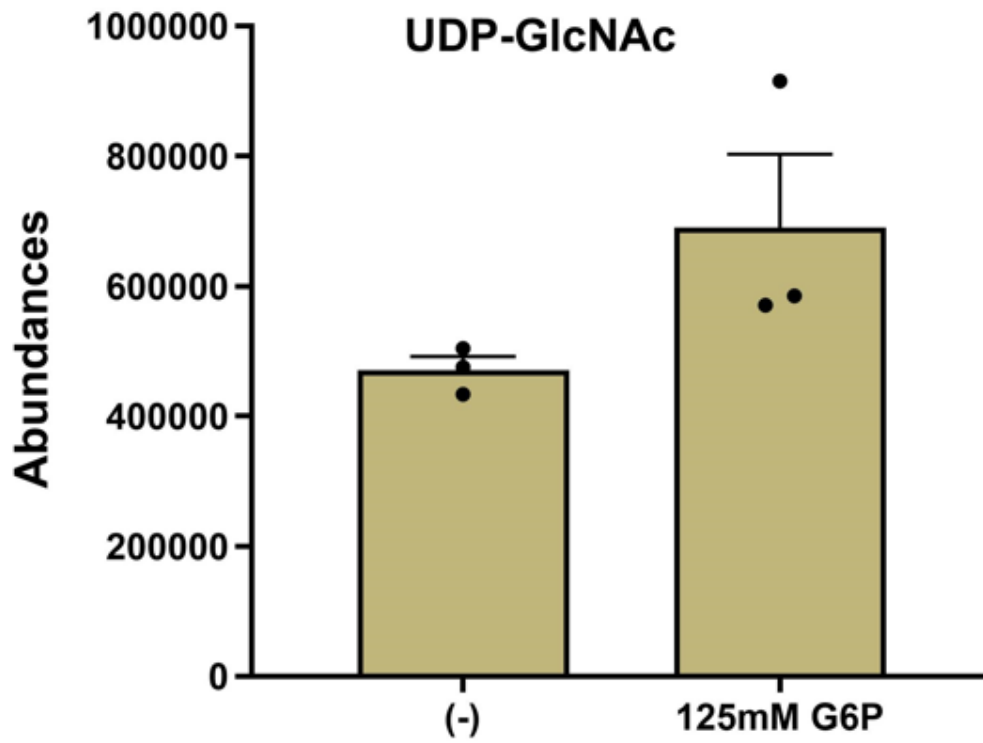


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779 Figure S2: Ability of  $\Delta pgi$  to grow on various carbon sources. A) Growth curves with M9  
 780 minimal media and the indicated sole carbon sources. SEM plotted. B) Serial dilutions of  
 781 overnight cultures grown in M9 + 0.2%CAA plated on M9 agar supplemented with the  
 782 indicated carbon sources and grown for 18 hours at 37°C. C) Growth curves with M9  
 783 minimal media, designated carbon sources, and 0.2% glucose. SEM plotted. D) Serial  
 784 dilutions of overnight cultures grown in M9 + 0.2%CAA plated on M9 agar supplemented  
 785 with the designated carbon sources and 0.2% glucose.  
 786



787

788 Figure S3: *In vitro* biochemical abundance of specified metabolites. UDP-GlcNAc levels  
789 measured with 125mM G1P addition. SEM plotted with 3 replicates displayed.

790

**A**

Model	Polar interactions with Glucosamine-1-P	Length of bond (Å) – some residues form multiple bonds with Glucosamine-1-P	Predicted Template Modeling (pTM) score (confidence in structure)	Interface Predicted Template Modeling (ipTM) score (confidence in interface)
0	Arg 330 Lys 348 Tyr 363 Asn 374 Asn 383 – this does not bind N in glucosamine Lys 389	3.0 3.5 2.9, 3.6, 3.7 2.1 3.2, 3.3, 3.4 2.8, 3.0, 3.2, 3.3	0.9556	0.9387
1	Arg 330 Lys 348 Asn 359 Tyr 363 Asn 383 Lys 389	2.9 2.9 3.2 2.5 2.9 2.4, 3.5	0.9558	0.9389
2	Arg 330 Tyr 363 Asn 374 Asn 383 Lys 389	2.9 2.4, 3.3, 3.4 2.0 3.2, 3.5 2.8	0.9553	0.9385
3	Arg 330 Lys 348 Tyr 363 Asn 374 Asn 383 – this binds N in glucosamine Lys 389	3.0 3.5 2.7, 2.9, 3.6 2.1 3.2, 3.3, 3.4 2.8, 3.0, 3.2, 3.3	0.9556	0.9388
4	Arg 330 Lys 348 Tyr 363 Asn 374 Asn 383 Lys 389	2.9, 3.4 2.8 2.5 1.9 3.5 2.8, 3.5	0.9554	0.9384

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**B**

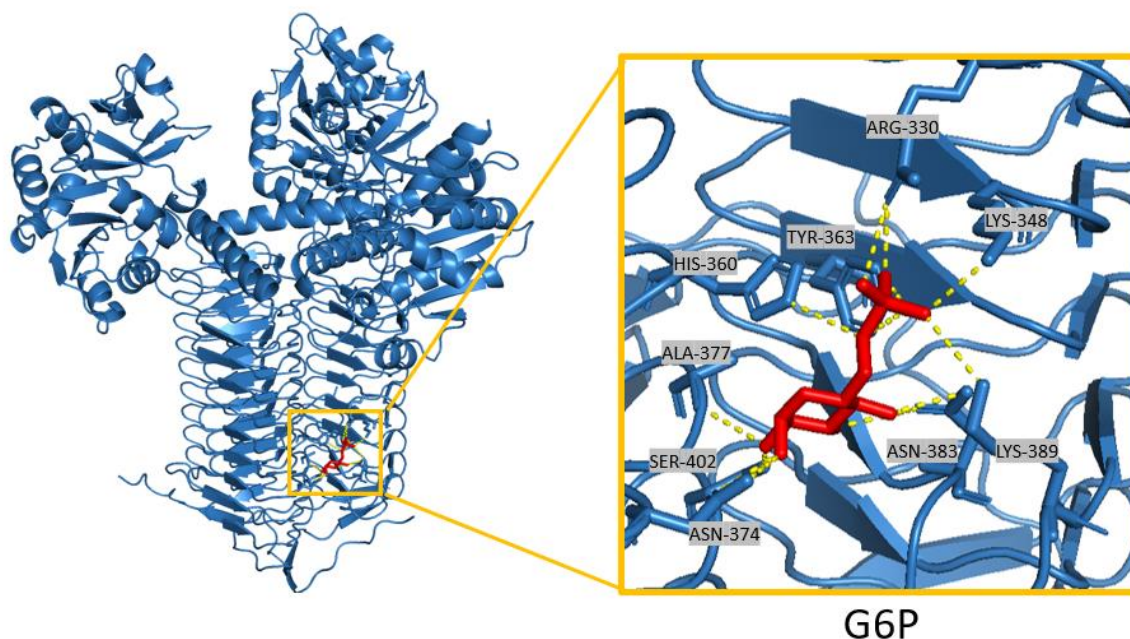
Model	Polar interactions with Glucose-1-P	Length of bond (Å) – some residues form multiple bonds with Glucose-1-P	Predicted Template Modeling (pTM) score (confidence in structure)	Interface Predicted Template Modeling (ipTM) score (confidence in interface)
0	Lys 12* Gly 13* Asp 102 Asn 224	2.9 3.1, 3.4 3.5 3.2	0.9553	0.9382
1	Leu 8* Ala 10* Lys 12* Lys 22	3.3 3.5 3.5 2.7, 3.6	0.9555	0.9385
2	Arg 330 His 360 Tyr 363 Asn 374 Asn 383 Lys 389	2.4, 3.4 3.4 2.0 2.8, 3.2 3.3, 3.3 2.7, 3.4	0.9548	0.9375
3	Lys 12* Gly 13* Lys 22 Gln 76 Asp 102 Asn 224	2.6 3.0, 3.4 2.6, 3.5 3.3 2.9 3.0	0.9552	0.9382
4	Arg 330 Lys 348 Asp 359 Tyr 363 Asn 374 Asn 383 Tyr 384* Lys 389	3.3 3.5 3.0 2.6, 3.1 3.0, 3.3 2.9, 3.3, 3.4 3.1 3.1, 3.5	0.9549	0.9376

C

Model	Polar interactions with G6P	Length of bond (Å) – some residues form multiple bonds with G6P	Predicted Template Modeling (pTM) score (confidence in structure)	Interface Predicted Template Modeling (ipTM) score (confidence in interface)
0	Asn 374 Ala 377 * Asn 383 His 360 Asn 359 Arg 330 Lys 348 Tyr 363	3.1, 2.8 3.3 2.6, 2.9, 3.0 3.5, 3.4 3.5 2.3, 3.4 3.0 2.5	0.9332	0.9083
1	Ala 377 * Asn 374 Ser 402 Asn 383 His 360 Tyr 363 Arg 330 Lys 348 Lys 389	3.3 2.9, 2.9 3.5 2.3, 3.0, 3.1 3.4 3.6, 2.7 3.4, 2.8 3.1 3.3	0.9342	0.9099
2	Asn 374 Asn 359 His 360 Arg 330 Tyr 363 Lys 348 Lys 389 Ser 402	2.2, 3.2, 3.6 2.8 3.5 2.6 1.9 3.5, 3.5 3.0 3.2	0.9335	0.9080
3	Asn 383 Tyr 363 Arg 330 Asn 374 Val 375 *	3.1 3.1, 3.4 2.3 2.4, 3.0 3.1	0.9336	0.9095
4	Asn 383 Tyr 363 Arg 330 Lys 389 Asn 359 Asn 374 Ala 377 *	2.9, 3.2 2.3 2.3, 3.5 3.3 3.0 2.8, 2.9 3.4	0.9336	0.9095

\*interaction with backbone, not side chain

D



794

795 Figure S4: Predicted molecular models of GlmU. A) Predicted models of VCH GlmU with  
796 glucosamine-1P with interacting residues denoted, length of bonds, pTM and ipTM  
797 scores. Model #3 was the model used above. B) Predicted models of VCH GlmU with  
798 glucose-1P with interacting residues denoted, length of bonds, pTM and ipTM scores.  
799 Model #4 was the model used above. Highlighted in green are the same interacting  
800 residues as the substrate GlcN-1P. C) Predicted models of VCH GlmU with glucose-6P  
801 with interacting residues denoted, length of bonds, pTM and ipTM scores. Model #1 was  
802 the model used above. Highlighted in green are the same interacting residues as the  
803 substrate GlcN-1P. D) Molecular modeling of GlmU binding glucose-6P (red) and the  
804 associated polar interactions.



**A**

CLUSTAL O(1.2.4) multiple sequence alignment

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sp|P9WHN3|GLMJ_MYCTU    MTFPGDTAVLVLAAGPGTRMRSDDPKVLTHTLAGRSHLSHVLAIAKLAPQRLIVVLGHDH  60
sp|P8ACC7|GLMJ_ECOLI    -MLNNMHSVVI LAAGKGRMYSDLPKVLTHTLAGKAPVQHVDAANELGAAHVHLVYGHGS  59
sp|Q9KIH7|GLMJ_VIBCH    ---MKFSTVILAAGKGRMHSNHPKVLTHTLAGKPHVHVITDTCNMLGAQNIHLVYGHGS  56
                          :.:***** :.:***** :.:***** :.:***** :.:*****

sp|P9WHN3|GLMJ_MYCTU    QRIAPLVGELADTLGRTIDVALQDRPLGTGHAVL CGLSALPDYAGHVVTSQREIPLDA  120
sp|P8ACC7|GLMJ_ECOLI    DLKKALK-----DDNLNMLVLAQELGTGHAMQQAAPFFADD - EDILMLYGDVPLTSV  111
sp|Q9KIH7|GLMJ_VIBCH    DQIQQAALA-----NIENMVLVLAQELGTGHAVDQASPHFQDD - EKILVLVYDVLTISE  108
                          :.:***** :.:***** :.:***** :.:*****

sp|P9WHN3|GLMJ_MYCTU    DTLADLIATHRAVSAAVTVLTTLDPPFGYGRILRTQDHEVMAIVEQTDATPSQREIREV  180
sp|P8ACC7|GLMJ_ECOLI    ETLQRLDQAKP--QGIGILLTVKLDPPTYGRITREN-GKVTGIVEHKDQDTEQRQIQEI  168
sp|Q9KIH7|GLMJ_VIBCH    DTIESLLEAQP--TDGIALLVLEDPYGRIVRKR-GPVVAIVEQKDASEEQKLIKEV   165
                          :*: * :. :. :*: * : * :* :* :* :* :* :* :* :* :* :* :* :* :* :*

sp|P9WHN3|GLMJ_MYCTU    NAGVYAFDIAALRSALSRLSNNAQQEYLYLTDVIAILRSDGQTVHSHVDDALVAGVIN  240
sp|P8ACC7|GLMJ_ECOLI    NTGILIANADMKRWLAKLTNNNAQGEYYITDIIALAYQEGREIVAVHPQRLSEVEGVIN  228
sp|Q9KIH7|GLMJ_VIBCH    NTGVLVATGRDLKRWLAGLNNAQGEYYLTDVIAAAHDEGRAVEAVHPSHSIEVEGVND  225
                          *: * :. :. :* :* :* :* :* :* :* :* :* :* :* :* :* :* :* :* :*

sp|P9WHN3|GLMJ_MYCTU    RVQLAEASELNRRVVAHQLAGVTVDPAITWIDVDVTIGRDVTIHPGTQLLGRTOIGG  300
sp|P8ACC7|GLMJ_ECOLI    RLQSLRLERVYQSEAEKLLLAGVMLRDPARFDLRLGTLHGRDVEIDTNVIEGNVTLGH  288
sp|Q9KIH7|GLMJ_VIBCH    RIQLARLERAFQARQAKLLAQVMLRDPARFDLRLGTLQCGSDVEIDVNVIIEGNVISGN  285
                          **:*: :. :. :* :* :* :* :* :* :* :* :* :* :* :* :* :* :* :* :*

sp|P9WHN3|GLMJ_MYCTU    RCVVGPDTLLTDVAVDGASVVR--THGSSSIIQDGAAGVPTVLRPGLTADGDKLGAFV  359
sp|P8ACC7|GLMJ_ECOLI    RVKIGTGCVIKNSVIGDCEISPYTVVEDANLAACTIGPFARLRPGAELLEGAVHGNFV  348
sp|Q9KIH7|GLMJ_VIBCH    NVVIGAGSILKDCIEDNTVIRPVSIEGATVGENCTVGFPTLRPGAELRDDAHVGNFV  345
                          :* :. :. :. :* :* :* :* :* :* :* :* :* :* :* :* :* :* :* :* :*

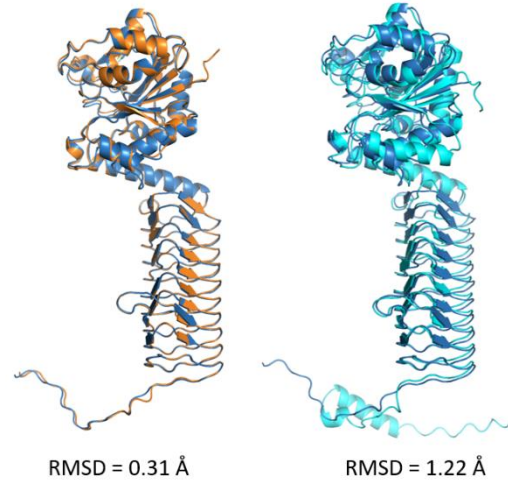
sp|P9WHN3|GLMJ_MYCTU    EVKNSTIGTGKVPHTLVYVDADIGEYSNIGASSVFVNYDGTSKRRTTVGSVRTGSDTH  419
sp|P8ACC7|GLMJ_ECOLI    EMKKNRLEKGSKAGHLYLGD AEI GDNWNVIGAGITTCNYDGANKFKTIIIGDDVFGSDTQ  408
sp|Q9KIH7|GLMJ_VIBCH    EMKNRLEKGSKAGHLYLGD AEI GDNWNVIGAGITTCNYDGANKFKTIIIGDDVFGSDCQ  405
                          *:* :. :* :* :* :* :* :* :* :* :* :* :* :* :* :* :* :* :* :* :*

sp|P9WHN3|GLMJ_MYCTU    FVAPVTIGDGAYTGAAGTVVREDVPPGALAVSAGPQRNIEHWQRKRGSPAAQASKRASE  479
sp|P8ACC7|GLMJ_ECOLI    LVAPVTVGKATIAAGTTVTRNVGENALAI SRVPQTKEGWRPVRVKKK-----  456
sp|Q9KIH7|GLMJ_VIBCH    LVAPVTIAGATIGAGTTLTKVVAEGELVITRAPERKIAGWQRPAKKK-----  453
                          :***** :* :* :* :* :* :* :* :* :* :* :* :* :* :* :* :* :* :* :*

sp|P9WHN3|GLMJ_MYCTU    MACQQPTQPPDADQTP  495
sp|P8ACC7|GLMJ_ECOLI    -----  456
sp|Q9KIH7|GLMJ_VIBCH    -----  453

```

**B**



**C**

	M. TB	E. coli	VCH
M. TB	100	38.68	40.04
E. coli	38.68	100	67.55
VCH	40.04	67.55	100

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Figure S5: Structural alignments of GlmU. A) Multiple amino-acid sequence alignments of *M. Tb*, *E. coli*, and *V. cholerae* with conserved residues denoted, using Clustal. B) GlmU monomer structural alignment. GlmU<sup>VC</sup> is blue, GlmU<sup>EC</sup> is depicted in orange, and GlmU<sup>Mtb</sup> is in teal. RMSD, root mean squared deviation, is a measure of how closely two alignments match; RMSD < 2.5 is a reasonable alignment. C) Percent Identity Matrix, created by Clustal2.1 shows alignment similarity across species.

Strain	Description	Source or Reference
WT*	Wild-type <i>V. cholerae</i> N16961 El Tor	Heidelberg JF, <i>et. al.</i> , 2000
MK1*	$\Delta pgi$ (vc0374)	Keller MK, <i>et. al.</i> , 2023
MK4	$\Delta pgi$ P <sub>tac</sub> - <i>pgi</i>	Keller MK, <i>et. al.</i> , 2023
MK12	$\Delta pgi$ P <sub>tac</sub> - <i>nagB</i> (vca1025)	This study
MK13*	$\Delta pgi$ $\Delta pgcA$ (vc2095)	This study
MK14	$\Delta pgi$ P <sub>tac</sub> - <i>pgcA</i>	This study
MK15	$\Delta pgi$ P <sub>tac</sub> - <i>glmS</i> (vc0487)	This study
MK16	$\Delta pgi$ pHL: <i>glmU</i> (vc2762)	This study
MK110	<i>E. coli</i> SM10 $\lambda$ pir conjugation strain	Ferrières L, <i>et. al.</i> , 2010
MK120	<i>E. coli</i> MFD pir conjugation strain	Ferrières L, <i>et. al.</i> , 2010
MK130	<i>E. coli</i> BL21 protein purification strain	Novagen

\* = checked via whole genome sequencing

814

815 Table S1: Strain list used in this study. \* = checked via whole genome sequencing

Plasmid	Description	Source or reference
pTD101	chromosomal lacZ insertion with IPTG induction	Obando MA, et. al., 2024
pTOX5	gene deletion construct	Lazarus JE, et. al., 2019
pET28a	6xHIS-SUMO tagged protein purification vector	Obando MA, et. al., 2024
pHL100mob	Non-integrative, high copy number plasmid with IPTG induction.	Mett H, et. al., 1980
pTD101 P <sub>tac</sub> - <i>nagB</i>	<i>nagB</i> overexpression with whole gene amplification using primers 7 and 8	This study
pTD101 P <sub>tac</sub> - <i>pgcA</i>	<i>pgcA</i> overexpression with whole gene amplification using primers 9 and 10	This study
pTD101 P <sub>tac</sub> - <i>glmS</i>	<i>glmS</i> overexpression with whole gene amplification using primers 11 and 12	This study
pHL100mob: <i>glmU</i>	<i>glmU</i> overexpression with whole gene amplification using primers 13 and 14	This study
pET28a <i>glmU</i>	GlmU protein purification strain	This study
pTOX <i>pgcA</i>	<i>pgcA</i> deletion by amplifying 500bp up and down stream of the gene using primers 15-18	This study

Primer description	Sequence (5'-3')	Number
pTD101fwd	ggcaaatattctgaaatgagctgt	1
pTD1010rev	cCAGATCTTAATTAAGGtgcttct	2
pTOXfwd	tcgctcgaaacctg	3
pTOXrev	gatcgagctcgagacg	4
pHL100fwd	cggataacaatttcacacagga	5
pHL100rev	gctgaaaatcttctcatccgc	6
<i>nagB</i> pTD101fwd	aacagaccatggaattcgagctcggtaccAGGAGGctgactgaATGAGACTTATCC	7
<i>nagB</i> pTD101rev	atgcctgcaggtcgactctagaggatccccTTAGAAGCCTAC	8
<i>pgcA</i> pTD101fwd	aacagaccatggaattcgagctcggtaccAGGAGGctgactgaATGGCTATGCACCCCT	9
<i>pgcA</i> pTD101rev	CGTG	10
<i>glmS</i> pTD101fwd	atgcctgcaggtcgactctagaggatccccTTATAAACCCGCGTCTTTAAACTTGGT	11
<i>glmS</i> pTD101rev	TTACG	12
<i>glmU</i> pHL100fwd	aacagaccatggaattcgagctcggtaccAGGAGGctgactgaATGTGTGGAATTGTT	13
<i>glmU</i> pHL100rev	GGTGC	14
<i>pgcA</i> up500fwd	atgcctgcaggtcgactctagaggatccccTACTCGACAGTTACCGCTTTAG	15
<i>pgcA</i> up500rv	aacagaccatggaattcgagctcggtaccAGGAGGctgactgaATGAAATTCAGTACG	16
<i>pgcA</i> dwn500fwd	GTAATTCTCG	17
<i>pgcA</i> dwn500rev	atgcctgcaggtcgactctagaggatccccTATTTCCTTTTCGCCGACGCTGC	18
<i>pgcA</i> flankfwd	ggcggggtttttctgtgatcacgtacgatCGAAAGGGATAGTCGTAAGCAAAGATGC	19
<i>pgcA</i> flankrev	TCATCATTACTCGAGTGCGCCGCATTAAGTGACATCCTTTCTTTAAATTT	20
<i>pgcA</i> internalfwd	CACATAAAATAAAACC	21
<i>pgcA</i> internalrev	TAATGCGGCCGCACTCGAGTAATAATGATGATGTGATGAAATGAATCAGG	22
MK chromolacZfw	CTTGCCTGCCGAGTTGAGT	23
MK chromolacZrev	CTGCCACTGGTAATGCGAGC	24

816

817 Table S2: Oligos used in this study.

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