

Lazar et al, Supplementary Information

Figure S1: Sequence alignment of the *bioQ* frameshift made via CRISPR. (A) CRISPR-Cas9 was used to generate frameshift mutants in *bioQ pyc::tn*. Candidate *bioQ* sequences were sequenced and sequences were aligned to the native *bioQ* sequence (top). Identical nucleotides are shaded in gray, while nucleotides different from the reference sequence are not shaded. *bioQ* mutant #16 has a single nucleotide insertion of A at the DSB site **(B)** *pyc::tn bioQ* #16 restores *bioB* induction to *pyc::tn*. Error bars are standard deviation of three biological replicates.

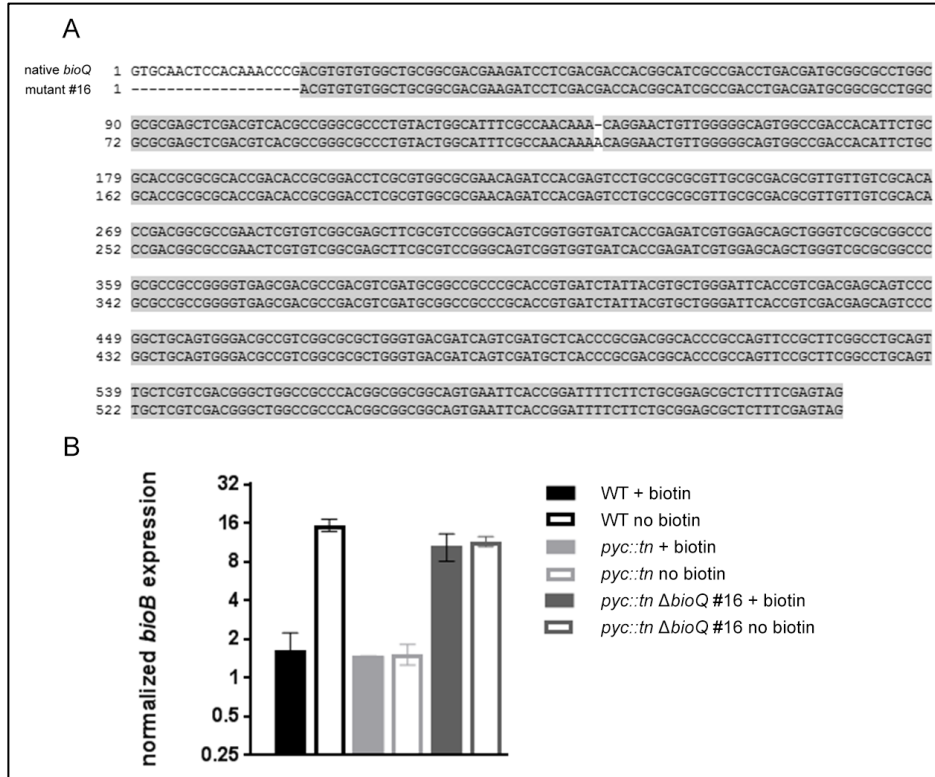


Figure S2. Primary amino acid sequence alignment of pyruvate carboxylases Rv2967c (*M. tuberculosis*) and MSMEG_2412 and 6648 (*M. smegmatis*). Sequence alignment was performed using the Clustal Omega web form (McWilliam *et al.*, 2013, Sievers *et al.*, 2011). Domains were identified by homology to the *R. etli* pyruvate carboxylase (St Maurice *et al.*, 2007). The biotin carboxylase (BC) domain is highlighted with a red line above the primary sequence, carboxytransferase (CT) with a blue line, and the biotin-binding (BCCP) with a green line. Biotin carboxylase catalytic residues (red arrows) were identified via homology with the *E. coli* biotin carboxylase, (Janiyani *et al.*, 2001) carboxytransferase catalytic residues (blue arrows) were identified via homology with the *S. aureus* pyruvate carboxylase (Yu *et al.*, 2009), and the biotin attachment residue (green arrow) was identified via the CDD database (Marchler-Bauer *et al.*, 2017). Transposon insertion sites are noted with black arrows and annotated with the strain number.



Table S1: Oligonucleotides used in this study

| Name | Sequence | Relevant Features |
|-------------|--------------------------|--|
| oNZL003 | GCCTTCTTGACGAGTTCTTCTGAG | sequencing of transposon insertions (F direction) |
| oNZL004 | CCGAGATAGGGTTGAGTGTGTTTC | sequencing of transposon insertions (R direction) |
| oCrBioQ-1F | CACCCTGGCATTTCGCCAACAAAC | crRNA deletion of <i>bioQ</i> |
| oCrBioQ-1R | AAACGTTTGTGGCGAAATGCCAG | crRNA deletion of <i>bioQ</i> |
| oCrBioQ-2F | CACCGAGATCGTGGAGCAGC | crRNA deletion of <i>bioQ</i> |
| oCrBioQ-2R | AAACGCTGCTCCACGATCTCGGTG | crRNA deletion of <i>bioQ</i> |
| oBioF-F | CACCCGACATCGTGATGAC | primer for qPCR amplification of <i>bioF</i> (F direction) |
| oBioF-R | CGGTGTCGAAGATGAAC | primer for qPCR amplification of <i>bioF</i> (R direction) |
| oBioF-taq | CACGACGCTGTGCAAAGCACTG | taqMan probe for qPCR quantitation of <i>bioF</i> |
| oBioA-F | CTGGACACGGTGTCTTC | primer for qPCR amplification of <i>bioA</i> (F direction) |
| oBioA-R | TCATCAACCGGTGCTTGG | primer for qPCR amplification of <i>bioA</i> (R direction) |
| oBioA-taq | TGGAGGTCGCGGTGAAGATG | taqMan probe for qPCR quantitation of <i>bioA</i> |
| oBioD-F | ACCGCATTGACCCTCGAAG | primer for qPCR amplification of <i>bioD</i> (F direction) |
| oBioD-R | ATCCAGTCTGCGTCAAAC | primer for qPCR amplification of <i>bioD</i> (R direction) |
| oBioD-taq | CGAACGCTTCGAGGCGTTGAG | taqMan probe for qPCR quantitation of <i>bioD</i> |
| oBioB-F | CATCATCAGCCTCAAGAC | primer for qPCR amplification of <i>bioB</i> (F direction) |
| oBioB-R | TTCGGTGGCACCGGTCTTTG | primer for qPCR amplification of <i>bioB</i> (R direction) |
| oBioB-taq | TGCCACTTCTGCTCGCAGT | taqMan probe for qPCR quantitation of <i>bioB</i> |
| oSigA-F | TGAGGTGACCGACGATCT | primer for qPCR amplification of <i>sigA</i> (F direction) |
| oSigA-R | GGCATCAGCTTCTTCTTCCT | primer for qPCR amplification of <i>sigA</i> (R direction) |
| oSigA-taq | AAGACACCGACCTGGAACCTCG | taqMan probe for qPCR quantitation of <i>sigA</i> |

Table S2: Plasmids used in this study

| Name | Relevant Features |
|---------------|---|
| pMSG419 | TET-ON dependent expression vector with a polylinker for N-terminal HA fusions <i>hygR oriM oriE</i> |
| pNZL035 | pMSG419 / MSMEG_2412 |
| pNZL036 | pMSG419 / MSMEG_2413 |
| pNZL037 | pMSG419 / MSMEG_2414 |
| pNZL106 | TET-ON dependent expression vector with a polylinker for N-terminal HA fusions <i>strepR oriM oriE</i> |
| pNZL035.strep | pNZL106 / MSMEG_2412 (pyruvate carboxylase) |
| pNZL065 | pNZL106 / MSMEG_6648 (other annotated <i>M. smegmatis</i> pyruvate carboxylase) |
| pNZL070 | pNZL106 / Rv2967c (<i>M. tuberculosis pyc</i>) |
| pNZL107 | pNZL106 / MSMEG_2412 biotin carboxylase (BC) domain (2412 residues 1-441) |
| pNZL108 | pNZL106 / MSMEG_2412 carboxytransferase (CT) domain (2412 residues 498-965) |
| pNZL109 | pNZL106 / MSMEG_2412 CT + biotin binding (BCCP) domain (2412 residues 498-1128) |
| pNZL154 | pNZL106 / MSMEG_2412 E288K biotin carboxylase catalytic site mutant |
| pNZL163 | pNZL106 / MSMEG_2412 K1093L biotin attachment site mutant |
| pNZL164 | pNZL106 / MSMEG_2412 K1093R biotin attachment site mutant |
| pNZL156 | pNZL106 / MSMEG_3188 (BioA) |
| pNZL159 | pNZL106 / MSMEG_3194 (BioB) |
| pAJF658 | TET-ON dependent expression vector with a DAS tag for Dual Control / hCas9 <i>hygR oriE int attP</i> |
| pAJF619 | tracrRNA parent plasmid <i>strepR oriE oriM</i> |
| pBioQ-1 | pAJF619 fused to oCrBioQ-1F/R via oligonucleotide ligation |
| pBioQ-2 | pAJF619 fused to oCrBioQ-2F/R via oligonucleotide ligation |

Table S3: Strains used in this study

| Strain number | Description | Genotype/Relevant Features | Reference |
|---------------|---|--|-----------------------|
| MGM8000 | WT | Wild Type <i>M. smegmatis</i> mc2155 | Laboratory collection |
| MGM8001 | m10 | Mc2155 <i>MSMEG_2412::tn</i> | this work |
| MGM8002 | m35 | Mc2155 <i>bioB::tn</i> | this work |
| MGM8003 | m43 | Mc2155 <i>bioF::tn</i> | this work |
| MGM8004 | m86 | Mc2155 <i>bioA::tn</i> | this work |
| MGM8005 | m119 | Mc2155 <i>MSMEG_2412::tn</i> | this work |
| MGM8006 | m134 | Mc2155 <i>MSMEG_2412::tn</i> | this work |
| MGM8007 | WT + EV | Mc2155 / pMSG419 | this work |
| MGM8008 | <i>pyc::tn</i> + EV | MGM8006 / pMSG419 | this work |
| MGM8009 | <i>bioA::tn</i> + EV | MGM8004 / pMSG419 | this work |
| MGM8010 | <i>bioB::tn</i> + EV | MGM8002 / pMSG419 | this work |
| MGM8011 | <i>pyc::tn</i> + pPyc | MGM8006 / pNZL035 | this work |
| MGM8012 | <i>pyc::tn</i> + p2413 | MGM8006 / pNZL036 | this work |
| MGM8013 | <i>pyc::tn</i> + p2414 | MGM8006 / pNZL037 | this work |
| MGM8019 | WT + EV | Mc2155 / pNZL106 | this work |
| MGM8020 | <i>pyc::tn</i> + EV | MGM8006 / pNZL106 | this work |
| MGM8021 | <i>bioA::tn</i> + EV | MGM8004 / pNZL106 | this work |
| MGM8023 | <i>pyc::tn</i> + pPyc | MGM8006 / pNZL035.strep | this work |
| MGM8024 | <i>pyc::tn</i> + p6648 | MGM8006/ pNZL065 | this work |
| MGM8025 | <i>pyc::tn</i> + pRv2967c | MGM8006/ pNZL070 | this work |
| MGM8026 | <i>pyc::tn</i> + pPyc (BC domain) | MGM8006 / pNZL107 | this work |
| MGM8027 | <i>pyc::tn</i> + pPyc (CT domain) | MGM8006 / pNZL108 | this work |
| MGM8028 | <i>pyc::tn</i> + pPyc (CT + BCCP domains) | MGM8006 / pNZL109 | this work |
| MGM8029 | <i>pyc::tn</i> + pPyc-E288K | MGM8006 / pNZL154 | this work |
| MGM8030 | <i>pyc::tn</i> + pPyc-K1093L | MGM8006 / pNZL163 | this work |
| MGM8031 | <i>pyc::tn</i> + pPyc-K1093R | MGM8006 / pNZL164 | this work |
| MGM8034 | <i>pyc::tn</i> + pBioA | MGM8006 / pNZL156 | this work |
| MGM8035 | <i>pyc::tn</i> + pBioB | MGM8006 / pNZL159 | this work |
| MGM8036 | <i>bioA::tn</i> + pBioA | MGM8004 / pNZL156 | this work |
| MGM8037 | <i>bioA::tn</i> + pBioB | MGM8004 / pNZL159 | this work |
| MGM8041 | <i>bioQ</i> mutant #16 | MGM8006 <i>bioQ</i> frameshift 138-139 A | this work |
| MGM6514 | Δ <i>pyc</i> | Mc2155 Δ <i>MSMEG_2412</i> (1-3384) | this work |
| MGM6516 | Δ <i>bioQ</i> | Mc2155 Δ <i>bioQ</i> (12-609) | this work |
| MGM6520 | <i>pyc::tn</i> / Δ <i>bioQ</i> | MGM8006 <i>bioQ::hyg</i> | this work |
| MGM6521 | Δ <i>pyc</i> + EV | MGM6514 / pNZL106 | this work |
| MGM6522 | Δ <i>pyc</i> + pNZL107 | MGM6514 / pNZL107 | this work |
| MGM6523 | Δ <i>pyc</i> + pNZL108 | MGM6514 / pNZL108 | this work |
| MGM6524 | Δ <i>pyc</i> + pNZL109 | MGM6514 / pNZL109 | this work |
| MGM6525 | Δ <i>pyc</i> + pNZL154 | MGM6514 / pNZL154 | this work |
| MGM6526 | Δ <i>pyc</i> + pNZL163 | MGM6514 / pNZL163 | this work |
| MGM6527 | Δ <i>pyc</i> + pNZL164 | MGM6514 / pNZL164 | this work |
| MGM6518 | <i>pyc::tn</i> + <i>B. subtilis</i> Pyc | MGM8005/pAJF841 | this work |