

Supplemental Figure 1. Structure of plasmid pUS116. Diagram made with SnapGene software.

n_etnH : **ATGAACGCACGCGATCTCGCAGGC****CGA**AGCTGGGGTGTAACACGGGCGATGCTGCAACTGTCT**CGA**TCCGTTTTG : 75
s_etnH : **ATGAACGCACGCGATCTCGCAGGC****CGT**AGCTGGGGTGTAACACGGGCGATGCTGCAACTGTCT**CGT**TCCGTTTTG : 75
n_etnH : CTCTCACTTAGTGACGGCTTAATCGTCTGGCGAATATTGGCCTG**TCC**GGACACAACGCCAAGCCGGAAGCTGTCA : 150
s_etnH : CTCTCACTTAGTGACGGCTTAATCGTCTGGCGAATATTGGCCTG**TCT**GGACACAACGCCAAGCCGGAAGCTGTCA : 150
n_etnH : CGCGAGGACGATGTGCAACCA**AGG**AGTATCGACGAATATCAGTGCGGGTCTTCCTCATCGATCCTGGACAATGCG : 225
s_etnH : CGCGAGGACGATGTGCAACCA**CGG**AGTATCGACGAATATCAGTGCGGGTCTTCCTCATCGATCCTGGACAATGCG : 225
n_etnH : ACG**TCC**GAACA**AGA**ACCGAAGATCTG**CCC**GTCAAAGGAAGTGGGCGCCCGAGCAGTCTGCTCTCG**CCC**TGGGTC : 300
s_etnH : ACG**TCC**GAACA**CGT**ACCGAAGATCTG**CCG**GTCAAAGGAAGTGGGCGCCCGAGCAGTCTGCTGTCTCG**CCG**TGGGTC : 300
n_etnH : AATGAAGAAGGAGCCGTT**CTA**AATGGCTGTT**AG**
s_etnH : AATGAAGAAGGAGCCGTT**CTA**AATGGCTGTT**TAA**

n_etnA : **ATGGCTGTTAGTCACATTCCACCGCAGCGGAAGGCGCTGTCCGAAACCGGACATTCCGGCTGGGTGACGCCAAAAGGCAAGCGT** : 84
s_etnA : **ATGGCTGTTAGTCACATTCCACCGCAGCGGAAGGCGCTGTCCGAAACCGGACATTCCGGCTGGGTGACGCCAAAAGGCAAGCGT** : 84
n_etnA : **CCC**ACCGAGTACGAAGATCTGACCGTAGGGCAGCAGTCGACCCCGGCGCAGTTCGCATTT**CGA**AGGCTGGCCGCT**CGA**TTTCGAC : 168
s_etnA : **CCG**ACCGAGTACGAAGATCTGACCGTAGGGCAGCAGTCGACCCCGGCGCAGTTCGCATTT**CGT**AGGCTGGCCGCT**CGT**TTTCGAC : 168
n_etnA : AACGGACGCGAC**CCCT**TACACCGAAGACTCGACGGTACTGCGCTCGTCTAACTGGTACGCGTATCGCGATCCGAACCAGACGATG : 252
s_etnA : AACGGACGCGAC**CCGT**TACACCGAAGACTCGACGGTACTGCGCTCGTCTAACTGGTACGCGTATCGCGATCCGAACCAGACGATG : 252
n_etnA : AACCGTCCGTACATCGCTGGCGTACACGAAGCTGAAAAAGCCCTCCAGAACACGTTCCCTCGGTGCGAGTGCGGGCGGCCTGTTC : 336
s_etnA : AACCGTCCGTACATCGCTGGCGTACACGAAGCTGAAAAAGCCCTCCAGAACACGTTCCCTCGGTGCGAGTGCGGGCGGCCTGTTC : 336
n_etnA : GGCTTCGCGAACCCTGAATGGGTGAGCAAGGCGCTCGCCAAGCACTACATGACGTAC**CCC**TTTCGTGAGTACGGCATGTTTCTT : 420
s_etnA : GGCTTCGCGAACCCTGAATGGGTGAGCAAGGCGCTCGCCAAGCACTACATGACGTAC**CCG**TTTCGTGAGTACGGCATGTTTCTT : 420
n_etnA : GCGCTGTGTTACGCAGAGCGCGAGGCACTTTCCGACACGGTGACGTTCTCTATCGTGTTTCGAGGCCGCGACAAACTGCGGCAC : 504
s_etnA : GCGCTGTGTTACGCAGAGCGCGAGGCACTTTCCGACACGGTGACGTTCTCTATCGTGTTTCGAGGCCGCGACAAACTGCGGCAC : 504
n_etnA : CTGCAAGATGTCGTCTACTACTCCTTCGAACCTGCCGAGGCGCACTCCGAATTCAGTGACGAGGAGTGCTTGCGGGTCTGGAAA : 588
s_etnA : CTGCAAGATGTCGTCTACTACTCCTTCGAACCTGCCGAGGCGCACTCCGAATTCAGTGACGAGGAGTGCTTGCGGGTCTGGAAA : 588
n_etnA : GAAGACCCGGTTTTGGCAGGGTGCTCGTGAGGCGATCGAGAACGTCATTGCGCTCGATGACTGGATGGAGATCGTCGTTGCCCTG : 672
s_etnA : GAAGACCCGGTTTTGGCAGGGTGCTCGTGAGGCGATCGAGAACGTCATTGCGCTCGATGACTGGATGGAGATCGTCGTTGCCCTG : 672
n_etnA : AACCTCTGCTTCGATCCGCTGTTCCGGTGAATTGGCGAAAGTCGAATACTTACC**CGA**TTTTCGCGGCGCGAACGGCGACCTGGTT : 756
s_etnA : AACCTCTGCTTCGATCCGCTGTTCCGGTGAATTGGCGAAAGTCGAATACTTACC**CGT**TTTTCGCGGCGCGAACGGCGACCTGGTT : 756
n_etnA : ACACCGTCGGTCATCGCCTCATCCGAG**GCC**GACACAGTGCAGCCCGGGCGTGGACAAAGGAACCTCATCCGCCATTGGGTGGG : 840
s_etnA : ACACCGTCGGTCATCGCCTCATCCGAG**GCG**GACACAGTGCAGCCCGGGCGTGGACAAAGGAACCTCATCCGCCATTGGGTGGG : 840
n_etnA : GAATCC**CGA**GTATGGCACCCACAACCTCGTCGATCATTGCAGCGTGGGTGGACAAGTGGAAACAGGTAATCGCTGAAAGCCTG**CGA** : 924
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n_etnA : CGCCTTCGC**CCC**CGTGTTCCT**CGA**GGTTCC**TAA** : 957
s_etnA : CGCCTTCGC**CCG**CGTGTTCCT**CGT**GGTTCC**TAA** : 957

n_etnB : **TTG**TCATCTGCCACG**CCC**GCTCGGGAT**AGA**ACGCAAGTCCGCGACACCGTCGGCATCTCGCTGATCGGGAGTTCA : 75
s_etnB : **ATG**TCATCTGCCACG**CCG**GCTCGGGAT**CGT**ACGCAAGTCCGCGACACCGTCGGCATCTCACTGATCGGGAGTTCA : 75
n_etnB : GAAACCAATGTCATCGTCGACATGGTTGCTGAGCTTCTC**CCC**GACGCCAAGATCACCGACAACGACGTGTTCTTC : 150
s_etnB : GAAACCAATGTCATCGTCGACATGGTTGCTGAGCTTCTC**CCG**GACGCCAAGATCACCGACAACGACGTGTTCTTC : 150
n_etnB : AAG**ATA**GAACGCGACGAAATGCTGAGCTTCGACATGGTTGAACTTAGCGAC**CGA**CTTGGCAAGCCGTATACGGTT : 225
s_etnB : AAG**ATT**GAACGCGACGAAATGCTGAGCTTCGACATGGTTGAACTTAGCGAC**CGT**CTTGGCAAGCCGTATACGGTT : 225
n_etnB : CATGACTTCCTCGTCAACATGACCAGTTACTACGGG**CGA**ATTGTCTGTAAGGATCACGAGATCGAGATCCACTCG : 300
s_etnB : CATGACTTCCTCGTCAACATGACCAGTTACTACGGG**CGT**ATTGTCTGTAAGGATCACGAGATCGAGATCCACTCG : 300
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s_etnB : GAGATCCTCCCGGAA**CGT**TTCCGCGACTAA : 330

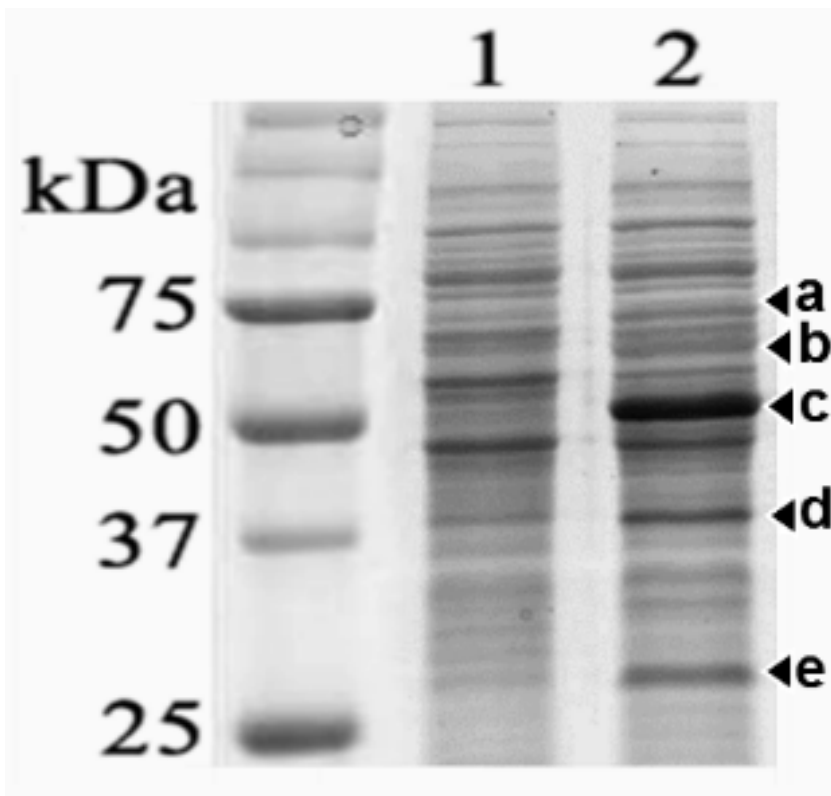
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n_etnC : TTTCCGACCCGCTACATCATC**CCC**CGAAGGGCAAGGATCCATTCCGGTCGATGCTGCGCGGTTACGCAGCGATGGAGACGGAA : 168
s_etnC : TTTCCGACCCGCTACATCATC**CCG**CGAAGGGCAAGGATCCATTCCGGTCGATGCTGCGCGGTTACGCAGCGATGGAGACGGAA : 168
n_etnC : AAGGACAACCGGGTCTACGGCGGTCTGGACAGCAACGTTTCGTTACCGCAACGCAACGTCGGCCGAGCCGCGCTTCATTGAAGGG : 252
s_etnC : AAGGACAACCGGGTCTACGGCGGTCTGGACAGCAACGTTTCGTTACCGCAACGCAACGTCGGCCGAGCCGCGCTTCATTGAAGGG : 252
n_etnC : ATGAAGTTCGGAATTCCAAGCTTCACCGACGCGGAGTACCAGGCAGTCTGCGGGTCAGGCTTTCTGATCGCATCCATGAAGAAC : 336
s_etnC : ATGAAGTTCGGAATTCCAAGCTTCACCGACGCGGAGTACCAGGCAGTCTGCGGGTCAGGCTTTCTGATCGCATCCATGAAGAAC : 336
n_etnC : CAGGAACTGCGCCAGGGCTACGCCGGCCAGATGCTCGACGAGGTTTCGTACACGCGAGATCGAAGTCGCCCTGCGGAAGTACTAC : 420
s_etnC : CAGGAACTGCGCCAGGGCTACGCCGGCCAGATGCTCGACGAGGTTTCGTACACGCGAGATCGAAGTCGCCCTGCGGAAGTACTAC : 420
n_etnC : CTGAAGAACTACCACGACCCGGCTGGCTTTGACATCGGCCAGATCGGTCTGGGTAATCACCCGATCGGCACCCTGGCACGGGCT : 504
s_etnC : CTGAAGAACTACCACGACCCGGCTGGCTTTGACATCGGCCAGATCGGTCTGGGTAATCACCCGATCGGCACCCTGGCACGGGCT : 504
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s_etnC : TCATTCCAGTCGTTCAACACCGGTGAT**CCG**TGGAGGTTTCGATGTGTCTGAACATCGTCCTCGAGACTGCCTACACGAAC**CCG** : 588
n_etnC : CTCGTGGTGGCCCTGCCCTCAGGTGCTGCCGTCAACGGAGAGCACGCGATGCCACTGCGTTCCTCTCGATCCAGTCTGACGAG : 672
s_etnC : CTCGTGGTGGCCCTGCCCTCAGGTGCTGCCGTCAACGGAGAGCACGCGATGCCACTGCGTTCCTCTCGATCCAGTCTGACGAG : 672
n_etnC : TCGCGGCACATGGCCAACGGCTATGGGACCTTGATGAGCGTTATTTCAGGAGCATGACAACCTGCCGTTCTGCAGGAATCGCTC : 756
s_etnC : TCGCGGCACATGGCCAACGGCTATGGGACCTTGATGAGCGTTATTTCAGGAGCATGACAACCTGCCGTTCTGCAGGAATCGCTC : 756
n_etnC : GACCGTCACTTCTGGCACCAGCATCAGTTCGATGGACACTCTGGTCGGCGTTCCTTTCCGAGTACTTCGCCGTGGAACGCCCTTGG : 840
s_etnC : GACCGTCACTTCTGGCACCAGCATCAGTTCGATGGACACTCTGGTCGGCGTTCCTTTCCGAGTACTTCGCCGTGGAACGCCCTTGG : 840
n_etnC : GCTTACAAAGATGTCTGGGAAGAGTGGTTCGTCGACGACTTCGTCGGCTCCTACATGAGCCGGCTCAGCCCCTCGGTCTCAAG : 924
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n_etnC : CCGCCGGCG**AGG**CTTGGTGACGTCGCCGCTATGTCAACGACATGCACCATTTCAGTGGCGATCGCGCTTGGCGCTATGTGGCCG : 1008
s_etnC : CCGCCGGCG**CGT**CTTGGTGACGTCGCCGCTATGTCAACGACATGCACCATTTCAGTGGCGATCGCGCTTGGCGCTATGTGGCCG : 1008
n_etnC : CTGAACTTCTGGCGGACCGACCCTATGGGTCCGGCAGACTACGAATGGTTTGAGAACCCTACCCGGGCTGGACCAAGTCCTAC : 1092
s_etnC : CTGAACTTCTGGCGGACCGACCCTATGGGTCCGGCAGACTACGAATGGTTTGAGAACCCTACCCGGGCTGGACCAAGTCCTAC : 1092
n_etnC : GGCGGTCTGTGGGACGCATTCCGCGATATGAGCGACCCGTCGTGGCG**CGA**ATCTTGTTCAGGAGTTGCCGTCACTGCCGCCG : 1176
s_etnC : GGCGGTCTGTGGGACGCATTCCGCGATATGAGCGACCCGTCGTGGCG**CGT**ATCTTGTTCAGGAGTTGCCGTCACTGCCGCCG : 1176
n_etnC : TTCTGCCAGGTCTGCCATGTTCCGTGCGTTATGCCGACATTGCACGCCCCGGAAACTCGCATCGTGTACGGCGAAGGCAAGAAG : 1260
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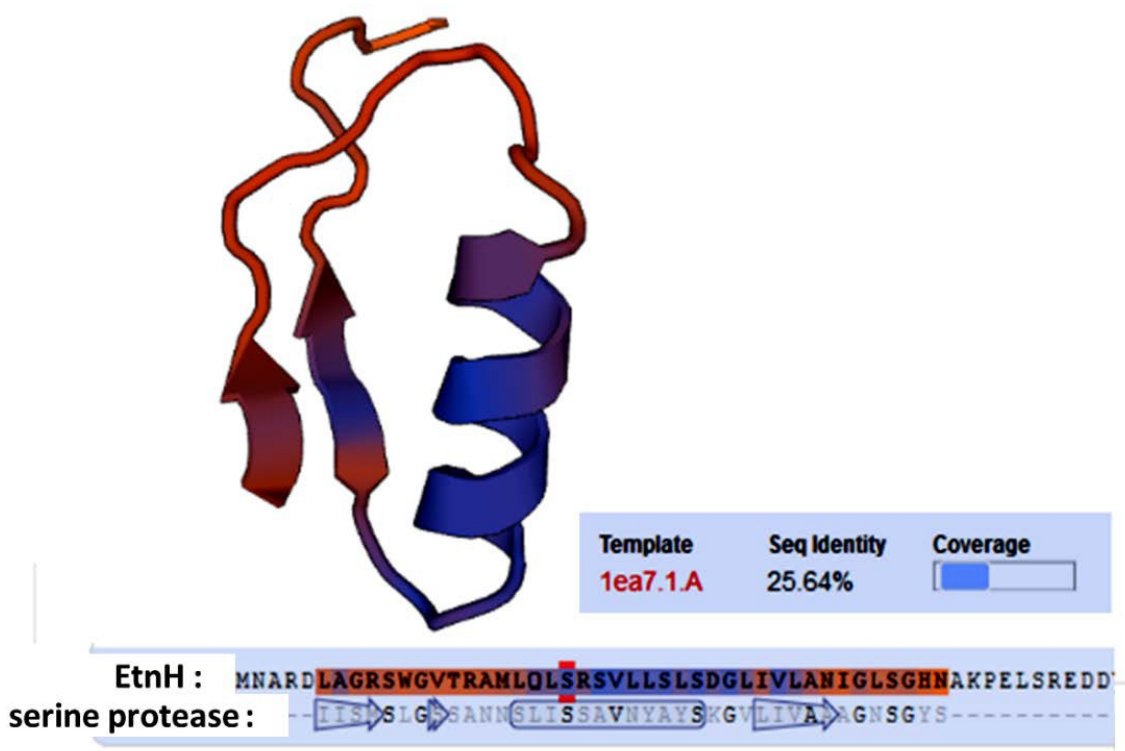
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s_etnD : AATGGCCGTTTCGTA AAAATACGGCTGCAAGCACGGCGGATGCAGCACCTGCCGTGCTCAGGTCGTGCGAAGGCGAATTCACCCAA : 168
n_etnD : TCCGACGGAACGTCGTTTTCCCTGAGCGACGCTGACCGCGACGCAGGTGTCGTGCTGCTCTGCTCGACATATGCAGACGGCGAC : 252
s_etnD : TCCGACGGAACGTCGTTTTCCCTGAGCGACGCTGACCGCGACGCAGGTGTCGTGCTGCTCTGCTCGACATATGCAGACGGCGAC : 252
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s_etnD : TTGGTCGTCGACGTCGGCGAGACCATGGCAGATCTGACCGAAGACGAGTACAACGCCGGCCAAGATATCGTCGAGTTCGTCGGT : 336
n_etnD : ACGGTCGACCGCATCGTCGACTACACGGCGGATATCAAGGGCATCGAGATCGCCTTGACGAGCCCTCAGCGATATCGTTCGTA : 420
s_etnD : ACGGTCGACCGCATCGTCGACTACACGGCGGATATCAAGGGCATCGAGATCGCCTTGACGAGCCGTCAGCGATTTCGTTCGTA : 420
n_etnD : CCCGGCCCAGTATGTGCAAGTCCTTGTCCC GGGATCCGACGACGCATGGCGGTCGTTCTCGATGGCCAACCGACCGAGCGACAAC : 504
s_etnD : CCCGGCCCAGTATGTGCAAGTCCTTGTCCC GGGATCCGACGACGCATGGCGGTCGTTCTCGATGGCCAACCGCCCGAGCGACAAC : 504
n_etnD : TCGAGGTGCACCTTGTAGTTCGCGTGATCCCCGATGGGCGGTTACCTCGCAGATCGGTACGACCATCTCCGCGGGGACAAGG : 588
s_etnD : TCGCGTTGCACCTTGTAGTTCGCGTGATCCCCGATGGGCGGTTACCTCGCAGATCGGTACGACCATCTCCGCGGGGACACGT : 588
n_etnD : CTGAACTTGCGCGGCCCATTTGGGGCAGTTTGAATTCGACTATCACACCGGCCGATAATCTTCATCGCGGGAGGTTCCGGGTATT : 672
s_etnD : CTGAACTTGCGCGGCCCATTTGGGGCAGTTTGAATTCGTCTGTCACACCGGCCGATTATTCTTCATCGCGGGAGGTTCCGGGTATT : 672
n_etnD : GCCCCGGTACTTTTCGATGCTCGCTGACCTTATCGAGCAGAATAATCAACGGCGAACGACGTTTCTCTACGGCGCGCGCACCGTG : 756
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n_etnD : GCTGACCTCCAATGCTGGACGAACTCGACAACTCTCAGATGAGCTGGACTGGTTCACTTTTATCCCTGCGCTGAGTCAGCCT : 840
s_etnD : GCTGACCTCCAATGCTGGACGAACTCGTCAACTCTCAGATGAGCTGGACTGGTTCACTTTTATCCCTGCGCTGAGTCAGCCT : 840
n_etnD : GACGATACCCCGTGGGACGGCGAAACCGGTTTGATCACCAGGTTTACCACCGAAACTTCCCGAGTGCCACGGACATGAGGCG : 924
s_etnD : GACGATACCCCGTGGGACGGCGAAACCGGTTTGATCACCAGGTTTACCACCGTAACTTCCCGAGTGCCACGGACATGAGGCG : 924
n_etnD : TACTTGTGTGGCCCCGCCTGGAATGATCGATGCAGCACTGGAGTCGCTGATCGCTAGCGGCTGTAAGGAGCGCCATATTTTCTTT : 1008
s_etnD : TATTTGTGTGGCCCCGCCTGGAATGATCGATGCAGCACTGGAGTCGCTGATCGCTAGCGGCTGTAAGGAGCGCCATATTTTCTTT : 1008
n_etnD : GACAGGTTCGTTCCGTCTGGATAG : 1032
s_etnD : GACCGTTTCGTTCCGTCTGGATAA : 1032

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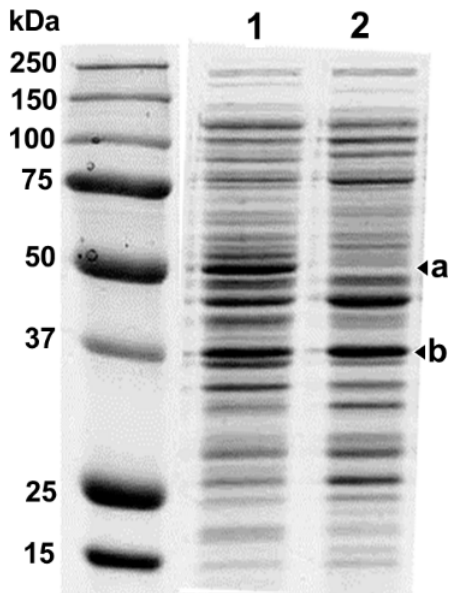
Supplemental Figure 2. Alignment of synthetic vs. native *etnHABCD* sequences. Native sequences are prefixed with a "n", synthetic sequences are prefixed with a "s". Rare codons are coloured in red (Arg), orange (Pro), green (Leu), or blue (Ile). The RBS's of all five genes were changed to AAGGAGG, with a spacing of six bases between the RBS and start codon.



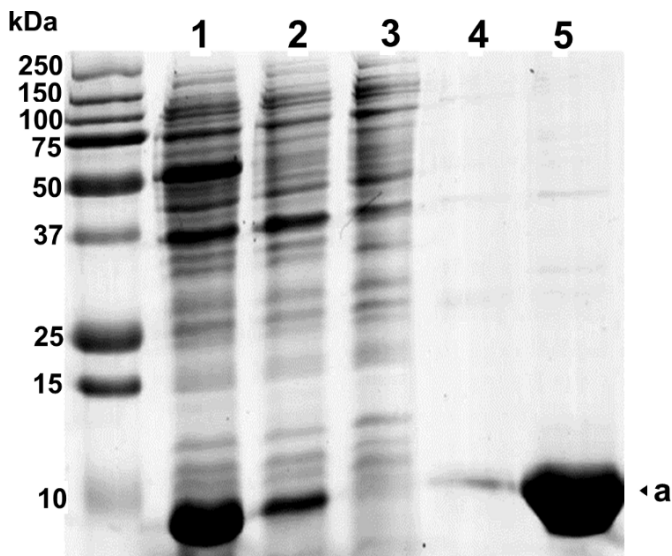
Supplemental Figure 3. Evidence of ethene MO expression in SDS-PAGE of *M. smegmatis* mc²-155 cell extracts. **Lane 1**, mc²-155(pUS116); **Lane 2**, mc²-155(pUS116-ETN). Both samples are from acetamide-induced cultures. Induced proteins unique to the mc²-155(pUS116-ETN) culture are labelled (a) to (e). the mc²-155(pUS116-ETN) culture are indicated by letters (a) to (e). Protein bands (c) and (d) correspond to the sizes of EtnC and EtnD, respectively.



Supplemental Figure 4. Structure modelling of EtnH of strain NBB4 using SWISSMODEL.



Supplemental Figure 5. SDS-PAGE comparison of soluble and total cell fractions of *E. coli* BL21 cells expressing *etnHABCD* genes. **Lane 1**, total cell lysate; **Lane 2**, soluble fraction. Label (a) shows the expected location of EtnC, and label (b) shows the expected location of EtnA. Note the presence of the putative EtnC band only in the total cell lysate, not the soluble fraction.



Supplemental Figure 6. Purification of His-tagged EtnB from BL21(DE3) cells.

Lane 1, Soluble fraction of whole cell lysate; Lane 2, nickel column wash step #1; Lane 3, nickel column wash step #2; Lane 4, nickel column elution step #1; Lane 5, nickel column elution step #2. The band labelled (a) corresponds to the predicted size of the 6xHis-tagged EtnB protein.