**Supporting information**

Table S1 Plasmids used in the study

| Plasmid | Description | Reference |
|---|---|---|
| pZElac | *E. coli* vector, amp$^R$ | [1] |
| pZElac-his | The pZElac inserted with the T5 promoter/lacO::6Xhis, amp$^R$ | [2] |
| pZElac_GDH | pZElac inserted with *E.coli gdhA* wild type, amp$^R$ | This work |
| pZElac_GDH F18 | pZElac inserted with *E.coli gdhA* F18 mutant, amp$^R$ | This work |
| pZElac_GDH F18TA | pZElac inserted with *E.coli gdhA* F18 with T195A mutation, amp$^R$ | This work |
| pZElac_GDH F18TV | pZElac inserted with *E.coli gdhA* F18 with T195V mutation, amp$^R$ | This work |
| pZElac_GDH F18TL | pZElac inserted with *E.coli gdhA* F18 with T195L mutation, amp$^R$ | This work |
| pZElac_GDH F18TF | pZElac inserted with *E.coli gdhA* F18 with T195F mutation, amp$^R$ | This work |
| pZElac_aspC | pZElac inserted with *E.coli aspC*, amp$^R$ | This work |
| pZElac_tyrB | pZElac inserted with *E.coli tyrB*, amp$^R$ | This work |
| pZElac_pheDH | pZElac inserted with *Thermoactinomyces intermedius pdh*, amp$^R$ | This work |
| pZElac_hisGDH | for his tag- *E.coli* GDH expression, amp$^R$ | This work |
| pZElac_hisF18 | for his tag- *E.coli* GDH F18 mutant expression, amp$^R$ | This work |
| pZElac_hisF18TA | for his tag- *E.coli* GDH F18 T195A expression, amp$^R$ | This work |
| KAVS library | pZElac vector harboring *E. coli gdhA* with K92, A166, V377 and S380 residues randomized by NNK codon | This work |

Reference:


1. Zhang, K., Li, H., Cho, K. M., Liao, J. C. 2010. Expanding metabolism for total biosynthesis of the nonnatural amino acid L-homoalanine. *Proc Natl Acad Sci U S A* 107: 6234-6239
2. Li, H., Liao, J. C. 2013. Engineering a cyanobacterium as the catalyst for the photosynthetic conversion of CO2 to 1,2-propanediol. *Microb Cell Fact* 12: 4

Table S2 Primers used in the study

| Primer | sequence (5'-3') |
|---|---|
| GDH Acc65I fwd | agaaaggtaccatggatcagacatattctctggagtc |
| GDH XbaI rev | gatgcctctagattaaatcacaccctgcgccagcatcg |
| aspC Acc65I fwd | gagaaaggtaccatgtttgagaacattaccgccgctc |
| aspC XbaI rev | gatgcctctagattacagcactgccacaatcgcttcg |
| tyrB Acc65I fwd | gagaaaggtaccatgtttcaaaaagttgacgcctacg |
| tyrB XbaI rev | gatgcctctagattacatcaccgcagcaaacgcctttgc |
| PheDH Acc65I fwd | gagaaaggtaccatgataagaatgggaagcatgaaaatc |
| PheDH XbaI rev | atgcctctagattacctccttgcgctgttgcgggg |
| T195A fwd | caacaataccgcctgcgtcttcgcgggtaagggcctttcatttggcg |
| T195A rev | cgccaaatgaaaggcccttacccgcgaagacgcaggcggtattgttg |
| T195L fwd | caacaataccgcctgcgtcttcctgggtaagggcctttcatttggcg |
| T195L rev | cgccaaatgaaaggcccttacccaggaagacgcaggcggtattgttg |
| T195V fwd | caacaataccgcctgcgtcttcgtgggtaagggcctttcatttggcg |
| T195V rev | cgccaaatgaaaggcccttacccacgaagacgcaggcggtattgttg |
| T195F fwd | caacaataccgcctgcgtcttcttcggtaagggcctttcatttggcg |
| T195F rev | cgccaaatgaaaggcccttaccgaagaagacgcaggcggtattgttg |
| K92 fwd | gcagttcagctctgccatcggcccgtacnnkggcggtatgcgcttccatccgtcagttaa |
| K92 rev | ttaactgacggatggaagcgcataccgccmnngtacgggccgatggcagagctgaactgc |
| A166 fwd | gccacctgggcgcggataccgacgttccgnnkggtgatatcggggttggtggtcgtgaag |
| A166 rev | cttcacgaccaccaaccccgatatcaccmnncggaacgtcggtatccgcgcccaggtggc |
| VS fwd | gtaaagcggctaatgctggtggcnnkgctacannkggcctggaaatggcacaaaacgctg |
| VS rev | cagcgttttgtgccatttccaggccmnntgtagcmnngccaccagcattagccgctttac |

**Cofactor specificity of GDH variants**

*E. coli* GDH is naturally NADPH-dependent. To determine the cofactor specificity of the engineered GDH variants toward the final target 2-OPBA, enzyme assays were performed using purified proteins (Figure S1). The results suggested that both GDH F18 and F18 T195A are highly NADPH-specific for this reaction. The NADH-dependent activity was very minimal.

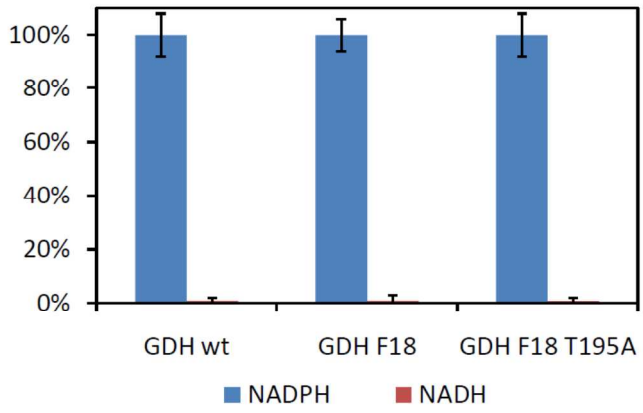


**Figure S1 Cofactor specificity of GDH variants.** 2-OPBA was the keto acid substrate used in the enzyme assays. The error bars represent the standard deviation of three repeats. (n=3)

**Performance of the second generation GDH variant, F18 T195A, in keto acid feeding experiments**

The test the enzyme's performance in converting the keto acid 2-OPBA to L-homophenylalanine inside the cells, GDH F18 T195A was overexpressed in *E. coli* and the keto acid feeding experiments were performed as described in the "methods" session. The results showed that F18 T195A produced higher level of L-homophenylalanine than the first generation variant F18. The increase was relatively small (~10%) but significant.

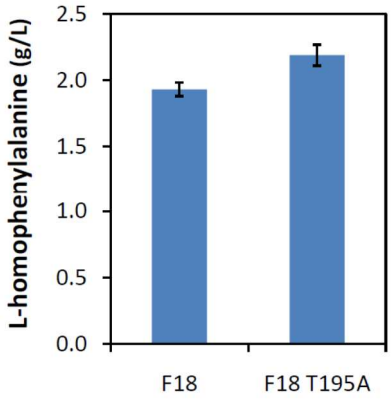


**Figure S2 Performance of F18 T195A GDH variant in the keto acid feeding experiments.** The error bars represent the standard deviation of three repeats. (n=3)