Supplementary Information

Biocatalytic reduction of α,β-unsaturated carboxylic acids to allylic alcohols
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**Fig. S1.** Screening of carboxylic acids using resting *E. coli* whole cells expressing either TpCAR (co-expressed with Sfp) or SrCAR (co-expressed with Sfp). Reaction contained 5 mM substrate, 20 mM D-glucose, 10 mM MgCl₂, 2% v/v DMSO and fresh resting *E. coli* cells containing overexpressed SrCAR at OD₆₀₀ nm = 30, all in NaPi (50 mM, pH7.5), incubated at 30 °C, 250 rpm for 18 h. SrCAR = *Segniliparus rugosus* carboxylic acid reductase. TpCAR = *Tsukamurella paurometabola* carboxylic acid reductase. Sfp = *Bacillus subtilis* phosphopantetheinyl transferase (sfp). Conversion values were determined from HPLC/GC-MS analyses.
CAR-GDH *in vitro* system monitored at 1h, 6h and 18h

**Fig. S2.** *In vitro* one-pot CAR-GDH biotransformation for the conversion of carboxylic acids to alcohol via intermediate aldehyde, monitored at 1h, 6h, 18h. In this system GDH was used as a bifunctional glucose oxidant (for NADPH recycling) and carbonyl reductase, catalysing reduction of aldehyde to the corresponding alcohol.

**DNA sequences of carboxylic acid reductases (CAR) and Sfp**

>**His-SrCAR gene sequence**  
ATGGGCAGCAAGCCATCATATCATCATCAGCAGCCGGCTTGGTGGCCGCACGGCAGCCATATGGCTAGCATGAGATGTCCGAGCAGCTACGAGACCAGGCCCGCCGACGAGCAGCCTCGGCAGCGCGTCGCGCGCTTGTCGCCATCGGCAAGCCGCGGCCGCTGTGCCGGACAAGGCCGTCGCCGAGCGCGACGCAGCAGGGTTTGCGCCTCGCGCAGCGGATCGAAGCCTTCCTCTCCGGCTACGGAGACCGCCGAGCCCTCGCCACAGCGCCCGACAGCCGATCGAAGGCCTTCCTCTCCGGCTACGGAGACCGCCGAGCCCTCGCCACAGCGCCCGACAGCCGATCGAAGGCCTTCCTCTCCGGCTACGGAGACCGCCGAGCCCTCGCCACAGCGCCCGACAGCCGATCGAAGGCCTTCCTCTCCGGCTACGGAGACCGCCGAGCCCTCGCCACAGCGCCCGACAGCCGATCGAAGGCCTTCCTCTCCGGCTACGGAGACCGCCGAGCCCTCGCCACAGCGCCCGACAGCCGATCGAAGGCCTTCCTCTCCGGCTACGGAGACCGCCGAGCCCTCGCCACAGCGCCCGACAGCCGATCGAAGGCCTTCCTCTCCGGCTACGGAGACCGCCGAGCCCTCGCCACAGCGCCCGACAGCCGATCGAAGGCCTTCCTCTCCGGCTACGGAGACCGCCGAGCCCTCGCCACAGCGCCCGACAGCCGATCGAAGGCCTTCCTCTCCGGCTACGGAGACCGCCGAGCCCTCGCCACAGCGCCCGACAGCCGATCGAAGGCCTTCCTCTCCGGCTACGGAGACCGCCGAGCCCTCGCCACAGCGCCCGACAGCCGATCGAAGGCCTTCCTCTCCGGCTACGGAGACCGCCGAGCCCTCGCCACAGCGCCCGACAGCCGATCGAAGGCCTTCCTCTCCGGCTACGGAGACCGCCGAGCCCTCGCCACAGCGCCCGACAGCCGATCGAAGGCCTTCCTCTCCGGCTACGGAGACCGCCGAGCCCTCGCCACAGCGCCCGACAGCCGATCGAAGGCCTTCCTCTCCGGCTACGGAGACCGCCGAGCCCTCGCCACAGCGCCCGACAGCCGATCGAAGGCCTTCCTCTCCGGCTACGGAGACCGCCGAGCCCTCGCCACAGCGCCCGACAGCCGATCGAAGGCCTTCCTCTCCGGCTACGGAGACCGCCGAGCCCTCGCCACAGCGCCCGACAGCCGATCGAAGGCCTTCCTCTCCGGCTACGGAGACCGCCGAGCCCTCGCCACAGCGCCCGACAGCCGATCGAAGGCCTTCCTCTCCGGCTACGGAGACCGCCGAGCCCTCGCCACAGCGCCCGACAGCCGATCGAAGGCCTTCCTCTCCGGCTACGGAGACCGCCGAGCCCTCGCCACAGCGCCCGACAGCCGATCGAAGGCCTTCCTCTCCGGCTACGGAGACCGCCGAGCCCTCGCCACAGCGCCCGACAGCCGATCGAAGGCCTTCCTCTCCGGCTACGGAGACCGCCGAGCCCTCGCCACAGCGCCCGACAGCCGATCGAAGGCCTTCCTCTCCGGCTACGGAGACCGCCGAGCCCTCGCCACAGCGCCCGACAGCCGATCGAAGGCCTTCCTCTCCGGCTACGGAGACCGCCGAGCCCTCGCCACAGCGCCCGACAGCCGATCGAAGGCCTTCCTCTCCGGCTACGGAGACCGCCGAGCCCTCGCCACAGCGCCCGACAGCCGATCGAAGGCCTTCCTCTCCGGCTACGGAGACCGCCGAGCCCTCGCCACAGCGCCCGACAGCCGATCGAAGGCCTTCCTCTCCGGCTACGGAGACCGCCGAGCCCTCGCCACAGCGCCCGACAGCCGATCGAAGGCCTTCCTCTCCGGCTACGGAGACCGCCGAGCCCTCGCCACAGCGCCCGACAGCCGATCGAAGGCCTTCCTCTCCGGCTACGGAGACCGCCGAGCCCTCGCCACAGCGCCCGACAGCCGATCGAAGGCCTTCCTCTCCGGCTACGGAGACCGCCGAGCCCTCGCCACAGCGCCCGACAGCCGATCGAAGGCCTTCCTCTCCGGCTACGGAGACCGCCGAGCCCTCGCCACAGCGCCCGACAGCCGATCGAAGGCCTTCCTCTCCGGCTACGGAGACCGCCGAGCCCTCGCCACAGCGCCCGACAGCCGATCGAAGGCCTTCCTCTCCGGCTACGGAGACCGCCGAGCCCTCGCCACAGCGCCCGACAGCCGATCGAAGGCCTTCCTCTCCGGCTACGGAGACCGCCGAGCCCTCGCCACAGCGCCCGACAGCCGATCGAAGGCCTTCCTCTCCGGCTACGGAGACCGCCGAGCCCTCGCCACAGCGCCCGACAGCCGATCGAAGGCCTTCCTCTCCGGCTACGGAGACCGCCGAGCCCTCGCCACAGCGCCCGACAGGC

OH

8a

OH

26a

OH

38a

O

O

F
>Bacillus subtilis sfp

ATGGGCAAGATTTACGGAATTTATATGGACCGCCCGCTTTCACAGGAAGAAAATGAACGGTTCATGACCTTCATATCACCTGAAAAACGGGAGAAATGCCGGAGATTTTATCATAAAGAAGATGCTCACCGCACCCCTGGGAGATGTGCTCGTTCGCTCAGTCATAAGCAGGCAGTATCAGTTGGACAAATCCGATATCCGGTTTAGCACGCAGGAATACGGGAAGCCGTGCATCCCTGATCTTCCCGACGCTCATTTCAACATTTCTCACTCCGGCCGCTGGGTCATTGGTGCGTTTGATTCACAGCCGATCGGCATAGATATCGAAAAAACGAAACGAGATCAGCCTTGAGATCGCCAAGCGCTTCTTTTCAAAAACAGAGTACAGCGACCTTTTAGCAAAAGACAGGACGAGCAGACAGACTATTTTTATCATCTATGGTCAATGAAAGAAAGCTTTATCAAACAGGAAGGCAAAGGCTTATCGCTTCCGCTTGATTCCTTTTCAGTGCGCCTGCATCAGGACGGACAAGTATCCATTGAGCTTCCGGACAGCCATTCCCCATGCTATATCAAAACGTATGAGGTCGATCCCGGCTACAAAATGGCTGTATGCGCCGCACACCCTGATTTCCCCGAGGATATCACAATGGTCTCGTACGAAGAGCTTTTA
Fig. S3. *In vitro* one-pot CAR_GDH cascade for reduction of benzofuran-2-carboxylic acid to benzofuran-2-methanol: HPLC traces from top to bottom: benzofuran-2-carboxylic acid standard, benzofuran-2-methanol standard, biotransformation samples taken at 6h using purified SrCAR+GDH. Retention times: starting material, benzofuran-2-carboxylic acid (3.70 min), benzofuran-2-methanol (4.02 min).
Fig. S4. In vitro one-pot CAR-GDH cascade for reduction of cinnamic acid to cinnamyl alcohol: HPLC traces of biotransformation reaction, samples taken at different time points (top to bottom: cinnamic acid standard, cinnamyl alcohol standard, biotransformation samples taken at 2 h, 4 h and 18 h). Retention times: starting material, cinnamic acid (3.80 min), cinnamaldehyde (4.91 min), cinnamyl alcohol (4.30 min).
Fig. S5. Biotransformation for conversion of benzofuran-3-carboxylic acid to the corresponding alcohol: GC traces of biotransformation reactions, showing: Row 1 = control reaction performed *E. coli* whole cells lacking the CAR<sup>sh</sup> system. Row 2 = whole-cell biotransformation reaction catalysed by recombinant *E. coli* whole cells expressing *Sr*CAR. Row 3 = *in vitro* one-pot CAR-GDH biotransformation.
Fig. S6. Biotransformation for conversion of benzothiophene-3-carboxylic acid to corresponding alcohol: GC traces of biotransformation reactions, showing: Row 1 = control reaction performed using *E. coli* whole cells lacking the CAR<sup>sfp</sup> system. Row 2 = whole-cell biotransformation reaction catalysed by recombinant *E. coli* whole cells expressing *SrCAR*. Row 3 = *in vitro* one-pot CAR-GDH biotransformation.
Fig. S7. Biotransformation for conversion of benzothiophene-2-carboxylic acid to corresponding alcohol:

GC traces of biotransformation reactions, showing: Row 1 = control reaction performed using E.coli whole-cells lacking the CAR® system. Row 2= whole-cell biotransformation reaction catalysed by recombinant E. coli whole cells expressing SrCAR. Row 3 = in vitro one-pot CAR-GDH biotransformation.
Fig. S8. Biotransformation for conversion of 2-naphthoic acid to the corresponding alcohol: GC traces of biotransformation reactions, showing: Row1 = control reaction performed using E. coli whole cells lacking the CARsfp system. Row2 = whole-cell biotransformation reaction catalysed by recombinant E. coli whole cells expressing SrCAR. Row = in vitro one-pot CAR-GDH biotransformation.
Fig. S9. Biotransformation for conversion of indole-2-carboxylic acid to the corresponding aldehyde/alcohol: GC traces of biotransformation reactions, showing: Row 1 = control reaction performed using *E. coli* whole cells lacking the CAR<sup>10</sup> system. Row 2 = whole-cell biotransformation reaction catalysed by recombinant *E. coli* whole cells expressing SrCAR. Row 3 = *in vitro* one-pot CAR-GDH biotransformation.
Fig. S10. Biotransformation for conversion of ferulic acid to the corresponding aldehyde/alcohol: GC traces of biotransformation reactions, showing: Row 1 = control reaction performed using *E.coli* whole cells lacking the CAR<sup>sp</sup> system. Row 2 = whole-cell biotransformation reaction catalysed by recombinant *E. coli* whole cells expressing SrCAR. Row 3 = *in vitro in vitro* one-pot CAR-GDH biotransformation. NB: *E. coli* whole cell lacking CAR<sup>sp</sup> system fully converted the acid to the decarboxylated product.
Fig. S11. Biotransformation for conversion of para-coumaric acid to the corresponding aldehyde/alcohol: GC traces of biotransformation reactions, showing: Row 1 = control reaction performed using E.coli whole cells lacking the CAR\textsuperscript{sfp} system. Row 2= whole-cell biotransformation reaction catalysed by recombinant E. coli whole cells expressing SrCAR. Row 3 = \textit{in vitro} one-pot CAR-GDH biotansformation. NB: E. coli whole cell lacking CAR\textsuperscript{sfp} system fully converted the acid to the decarboxylated product.
Fig. S12. Biotransformation for conversion of beta-methylcinnamic acid to the corresponding alcohol: GC traces of biotransformation reactions, showing: Row 1 = control reaction performed using *E. coli* whole cells lacking the CAR<sup>sfp</sup> system. Row 2 = whole-cell biotransformation reaction catalysed by recombinant *E. coli* whole cells expressing SrCAR. Row 3 = *in vitro* *in vitro* one-pot CAR-GDH biotransformation.
Fig. S13. Biotransformation for conversion of alpha-fluorocinnamic acid to the corresponding alcohol: GC traces of biotransformation reactions, showing: Row 1 = control reaction performed using \textit{E. coli} whole cells lacking the \textit{CAR}^{sfp} system. Row 2 = whole-cell biotransformation reaction catalysed by recombinant \textit{E. coli} whole cells expressing \textit{SrCAR}. Row 3 = \textit{in vitro} one-pot \textit{CAR-GDH} biotransformation.
**Fig. S14.** Biotransformation for conversion of alpha-methylcinnamic acid to the corresponding alcohol: GC traces of biotransformation reactions, showing: Row 1 = control reaction performed using *E. coli* whole cells lacking the CAR<sup>ep</sup> system. Row 2 = whole-cell biotransformation reaction catalysed by recombinant *E. coli* whole cells expressing SrCAR. Row 3 = *in vitro* one-pot CAR-GDH biotransformation.
Fig. S15. Biotransformation for conversion of p-methylcinnamic acid to the corresponding alcohol: GC traces of biotransformation reactions, showing: Row 1 = control reaction performed using E.coli whole cells lacking the CAR<sup>sfp</sup> system. Row 2= whole-cell biotransformation reaction catalysed by recombinant E. coli whole cells expressing SrCAR. Row 3 = in vitro one-pot CAR-GDH biotransformation.
Fig. S16. Biotransformation for conversion of 4-methylcinnamic acid to the corresponding alcohol: GC-MS trace of biotransformation reactions, showing. Row 1 = whole-cell biotransformation reaction catalysed by recombinant *E. coli* whole cells expressing SrCAR. Rows 2-4 = mass spectra extracted from biotransformation trace, showing products and intermediates.