Supplementary Material

Title

Rerouting phytosterol degradation pathway for directed androst-1,4-diene-3,17-dione microbial bioconversion

Authors and affiliation:

Xia Ke [1, 2], Jia-Hao Cui [1, 2], Qi-Jie Ren [1, 2], Tong Zheng[1, 2], Xin-Xin Wang [1, 2], Yu-Guo Zheng[1, 2], Zhiqiang Liu [1, 2*]

1 National and Local Joint Engineering Research Center for Biomanufacturing of Chiral Chemicals, Zhejiang University of Technology, Hangzhou 310014, People's Republic of China

2 Key Laboratory of Bioorganic Synthesis of Zhejiang Province, College of Biotechnology and Bioengineering, Zhejiang University of Technology, Hangzhou 310014, People's Republic of China

Table S1 Sequence of selected promoter and RBS used for the overexpression of rate-limiting enzymes.

Promoters	RBS	**Sequence**
SMYC	AGGAGG	GGATCGTCGGCACCGTCACGGCCGTGGGAGGCGGCA CGATCCGCGACGTGATGATCGGCCGCATCCCCACGGT GCTGCGCAGTGAGCTCTACGCCATCCCGGCGTTGATC TGTGCGTTCGCACGCACAGGCCCGGTGTGAGAAGGG TCTCTGACGAGCGGGAGAACCCACCCGGGGTGGGCG AGTTTGTCCTGCGTGTGCTCGGTCGAGTAGGCTCTGG GAGTACCCGTGTGTACGACCAGCACGGCATACATCA TTTCGACGCCGAGAGATTCGCCGCCCGAAATGAGCA CGATCCGCATGCTTAATTAAGGAGGTATCTCCATG
G13	AGGAGG	GATCGCCACTAGCGCCGCGGTCGGAATCAGCGCACT GGCCGCCGGAGCGGTCAGCTACCTGTTGCTGCGCCGC TTCGCTTTCTAGCACCGACGTCCCGGCGCCAGCAGCT ACCTCTCGAGCAAACGTTTTGCGCGCCCGAAAAATA AGTCGTTGCAGAACTTTCATGAATTAGGCCTTGCTGC GCCCAGGCTCCAGTAGTAGAAATGGAGTCACGGCAG CCCGGTGAAGCCAAGGTCGAACCGGAAGAGAAGGTT CGTCCTCCCGACCCGGGCACCCAGCACGGCCCCCGG

		AACCCACGCGGAGTCATAGCCGCGATAATGGCAGAA
		GTGTTGCGGGCCTGCGTAATTGCGAAATTCAGATGGT
		GCCGACGGCCCTTTGGGTGGGGCTGCAGCCAGAAGG
		GTCGCAAAAGCGCCGAGGCCAACCCACGCAGCCACA
		AATGCACGCTTGGTAACCGAAGGAGGTATCTCCATG
MOP	AGGAGG	GGCTCTAGCGCCGATGGTAGTGTGGGGTCTCCCCATG
		CGAGAGTAGGGAACTGCCAGGCATCAAATAAAACGA
		AAGGCTCAGTCGAAAGGCTGGGCCTTTCGTTTTATCT
		GTTGTTTGTCGGTGAACGCTCTCCTGAGTAGGACAAA
		TCCGCCGGGAGCGGATTTGAACGTTGCGAAGCAACG
		GCCCGGAGGGTGGCGGGCAGGACGCCCGCCATAAAC
		TGCCAGGCATCAAATTAAGCAGAAGGCCATCCTGAC
		GGATGGCCTTTTTGCGTTTCTACAAACTCTTCCTGTCG
		TCATATCTAGACCAGGCTTGACACTTTATGCTTCCGG
		CTCGTATAATGTGTGGAATTGTGAGCGCTCACAATTC
		GGATCCAGCGATGTCGACAGGAGGATTCACCATG
L1	AGGGAGA	CGATGATAAGCGGTCAAACATGAGAATTCGCGGCCG
		CATAATACGACTCACTATAGGGATCTTAATTAAGGCG
		CCTCATGTTCTTTCCTGCGTTATCCCCTGATTCTGTGG
		ATAACCGTATTACCGCCTTTGAGTGAGCTGATACCGC
		TCGCCGCAGCCGAACGACCGAGCGCAGCGAGTCAGT
		GAGCGAGGAAGCGGAAGAGCGCCCAATACGCAAAC
		CGCCTCTCCCAGATCTGATATCGCTAGAGGAAACAGC
		TATGACCATGATTACGCCAAGCTTGCATGCCTGCAGC
		TAGGGCACCAATTTGCGATTAGGGCTTGACAGCCACC
		CGGCCAGTAGTGCATTCTTGTGTCACCGCAGCAGCAA
		GGCGGTAGGCGGATCCGAGAGGATCGTGCCGGTGCC
		GGTGAAAATCCGGCGGCAAGATTCTCCGGTTTGACA
		GCCACCCGGTTATCGGGTAAGCTGCAAGCATCACCA
		ACTTGGACGGGAAAGGGAGATCGTCCATG
L2	AGGAGG	CGATGATAAGCGGTCAAACATGAGAATTCGCGGCCG
		CATAATACGACTCACTATAGGGATCTTAATTAAGGCG
		CCTCATGTTCTTTCCTGCGTTATCCCCTGATTCTGTGG
		ATAACCGTATTACCGCCTTTGAGTGAGCTGATACCGC
		TCGCCGCAGCCGAACGACCGAGCGCAGCGAGTCAGT
		GAGCGAGGAAGCGGAAGAGCGCCCAATACGCAAAC
		CGCCTCTCCCAGATCTGATATCGCTAGAGGAAACAGC
		TATGACCATGATTACGCCAAGCTTGCATGCCTGCAGC
		TAGGGCACCAATTTGCGATTAGGGCTTGACAGCCACC
		CGGCCAGTAGTGCATTCTTGTGTCACCGCAGCAGCAA
		GGCGGTAGGCGGATCCGAGAGGATCGTGCCGGTGCC
		GGTGAAGAGAGATTCGCCGCCCGAAATGAGCACGAT
		CCGCATGCTTAATTAAGGAGGTATCTCCATG

Table S2 Primers used in present study

Primers	Sequence	Application
Genetic deletion		
A1-U-F	aatgccgatatcttaattaaacgtcgggcatatcgtgatcgtcg	*kshA1-up*
A1-U-R	ggtgggcctcccgtatctggtc	
A1-D-F	ccagatacgggaggcccacctgaccacgcatgacgagcgg	*kshA1-down*
A1-D-R	tgaattggtaccgcggccgcaagaatccgagccacctgttcgc	
pR-U-F	aatgccgatatcttaattaaccgatgcgggcgttgggttc	*opccR-up*
pR-U-R	caggccacacctatcgtggtgacg	
pR-D-F	gtgtggcctgggtcgtctgtgtggcggcgtacg	*opccR-down*
pR-D-R	tgaattggtaccgcggccgcgtcttcgccaccgtgtccgatgg	
un-U-F	aaccgtattcctttaattaaagcacacgcgcccagacc	*cdun-up*
un-U-R	tctctctgcgatttcggcgcagtacgacgcgctgcgcc	
un-D-F	gcgccgaaatcgcagagagaatg	*cdun-down*
un-D-R	tgaattggtaccgcggccgctcctggaaaagcgcgatcccg	
al-U-F	aaccgtattcctttaattaaagttcgacggcgagttcctcg	*sal-up*
al-U-R	gaattgcacccacacctcgtcggtttacgctcccgtcagcc	
al-D-F	acgaggtgtgggtgcaattctcac	*sal-down*
al-D-R	tgaattggtaccgcggccgcagcgttgatcagaaaccagagtctttgc	
Quantitative-PCR		
q-*hsd4A*-F	tcaagagcccatgagctcggtgg	*q-hsd4A*
q-*hsd4A*-R	atgaacgacaacccgatcgacctg	
q-*sal*-F	ttgggtttgcgtggtgacgcag	*q-sal*
q-*sal*-R	tcaatccccctccaggacgagag	
q-o*pccR*-F	ctaccagtgcacacccggaatcg	*q-opccR*
q-*opccR*-R	atggcccgcatgcattatgtcg	
q-*choM2*-F	ttgctgacaagacggcggttcc	*q-choM2*
q-*choM2*-R	gatcgcctcgatattgcgttcggc	
q-*kstD1*-F	ggaatctacgttcgcgaggccg	*q-kstD1*

q-*kstD1*-R	gcgctgatacttggtgcgcatct	
q-16s rRNA-F	tcacgaacaacgcgacaaac	*q-16s rRNA*
q-16s rRNA-R	gcggtaatacgtagggtccg	
Overexpression		
261-GFP-hsp60-F	caatggccaagacaattgcggatccatggtctcgaagggcgagga	*pMV261-*
261-GFP-hsp60-R	cagtcgatcgtacgctagttaactcacttgtacagctcgtccatgcccag	*hsp60-eGFP*
261-GFP-smyc-F	gtaccagatctttaaatctagaggatcgtcggcaccgtcac	*pMV261-*
261-GFP-smyc-R	cctcgcccttcgagaccatggagatacctccttaattaagcatgcgg	*smyc- eGFP*
261-GFP-g13-F	taccagatctttaaatctagagatcgccactagcgc	*pMV261-*
261-GFP-g13-R	cctcgcccttcgagaccatggagatacctccttcggttaccaagc	*g13- eGFP*
261-GFP-mop-F	taccagatctttaaatctagaggctctagcgccgatggtagt	*pMV261-*
261-GFP-mop-R	cctcgcccttcgagaccatggtgaatcctcctgtcgacatcgc	*mop- eGFP*
261-GFP-L1-F	cggtaccagatctttaaatctagacgatgataagcggtcaaac	*pMV261-L1-*
261-GFP-L1-R	tcctcgcccttcgagaccatggacgatctccctttcc	*eGFP*
261-GFP-L2-F	taccagatctttaaatctagaccgatgataagcggtcaaacatgag	*pMV261-L2-*
261-GFP-L2-R	cctcgcccttcgagaccatggagatacctccttaattaagcatgc	*eGFP*
306-GFP-smyc-F	aacgcgtgcggccgctctagaggatcgtcggcaccgtcac	*pMV306-*
306-GFP-smyc-R	gtcgatcgtacgctagttaactcacttgtacagctcgtccatgccca	*smyc- eGFP*
306-GFP-g13-F	aacgcgtgcggccgctctagagatcgccactagcgccgc	*pMV306-*
306-GFP-g13-R	gtcgatcgtacgctagttaactcacttgtacagctcgtccatgccca	*g13- eGFP*
306-GFP-mop-F	caacgcgtgcggccgctctagaggctctagcgccgatggtagt	*pMV306-*
306-GFP-mop-R	gtcgatcgtacgctagttaactcacttgtacagctcgtccatgccca	*mop- eGFP*
306-GFP-L1-F	aacgcgtgcggccgctctagacgatgataagcggtcaaacatgagaattcg	*pMV306-L1-*
306-GFP-L1-R	gtcgatcgtacgctagttaactcacttgtacagctcgtccatgccca	*eGFP*
306-GFP-L2-F	aacgcgtgcggccgctctagacgatgataagcggtcaaacatgagaattcg	*pMV306-L2-*
306-GFP-L2-R	gtcgatcgtacgctagttaactcacttgtacagctcgtccatgccca	*eGFP*
261-choM2-smyc-F	attaaggaggtatctccttgctgacaagacggcggttc	*pMV261-*

261-choM2-smyc-R	cagtcgatcgtacgctagttaacctaacgccggcctgagatgatc	*smyc-choM2*
261-kstD1-smyc-F	attaaggaggtatctccatgactgaacaggactacagtgtctttg	*pMV261-*
261-kstD1-smyc-R	agtcgatcgtacgctagttaactcaggcctttccagcgagatgc	*smyc-kstD1*
261-choM2-hsp60-F	tgcggatccagctgcagaattcatgctgacaagacggcggttcc	*pMV261-*
261-choM2- hsp60-R	acgtcgacatcgataagcttctagatcgcctcgatattgcgttcggc	*hsp60-choM2*
261-kstD1-hsp60-F	cggatccagctgcagaattcgtgtggattccgaacaacgaggttct	*pMV261-*
261-kstD1-hsp60-R	acgtcgacatcgataagctttcaggcctttccagcgagatgca	*hsp60- kstD1*
261-choM2-g13-F	ccgaaggaggtatctccttgctgacaagacggcggttc	*pMV261-*
261-choM2-g13-R	gcagtcgatcgtacgctagttaacctaacgccggcctgagatgatcgc	*g13-choM2*
261-kstD1-g13-F	aaccgaaggaggtatctccatgactgaacaggactacagtgtctttg	*pMV261-*
261-kstD1-g13-R	cagtcgatcgtacgctagttaactcaggcctttccagcgagatgc	*g13-kstD1*
261-choM2-mop-F	cgacaggaggattcaccttgctgacaagacggcggttcct	*pMV261-*
261-choM2-mop-R	cagtcgatcgtacgctagttaacctaacgccggcctgagatgatcgc	*mop-choM2*
261-kstD1-mop-F	gtcgacaggaggattcaccatgactgaacaggactacagtgtctttg	*pMV261-*
261-kstD1-mop-R	cagtcgatcgtacgctagttaactcaggcctttccagcgagatgc	*mop-kstD1*
306-hsd4A-L2-F	attaaggaggtatctccatgaacgacaacccgatcgacctg	*pMV306-L2-*
306-hsd4A-L2-R	gtcgatcgtacgctagttaactcaagagcccatgagctcggtg	*hsd4A*
306-choM2-L2-F	attaaggaggtatctccttgctgacaagacggcggttc	*pMV306-L2-*
306-choM2-L2-R	gtcgatcgtacgctagttaacctaacgccggcctgagatgatc	*choM2*
306-kstD1-L2-F	attaaggaggtatctccatgactgaacaggactacagtgtctttg	*pMV306-L2-*
306-kstD1-L2-R	gtcgatcgtacgctagttaactcaggcctttccagcgagatg	*kstD1*

Figure S1 Time course accumulation of ADD in the biotransformation system using resting cells of *M. neoaurum* NRRL B-3805 as the catalysts with/without *kshA1* deletion.

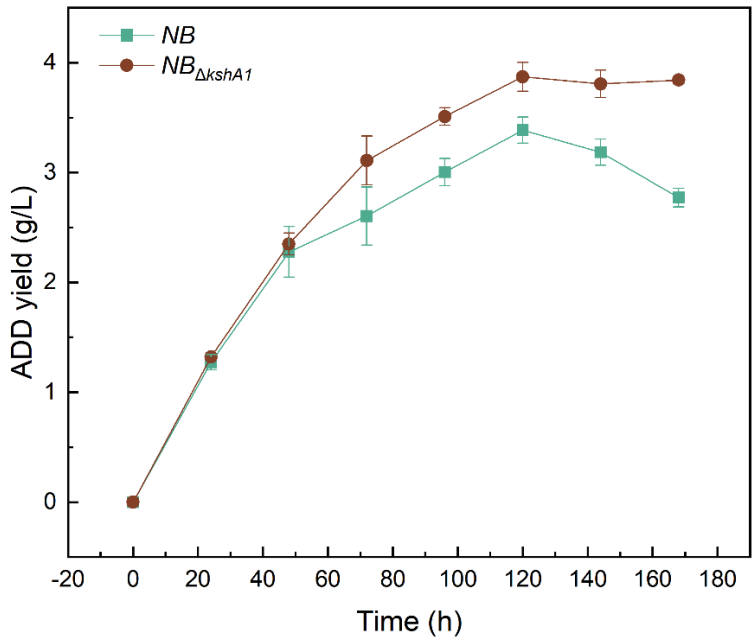

Figure S2 Amino acids sequence alignment between C-terminal of OpccR and Cdun.

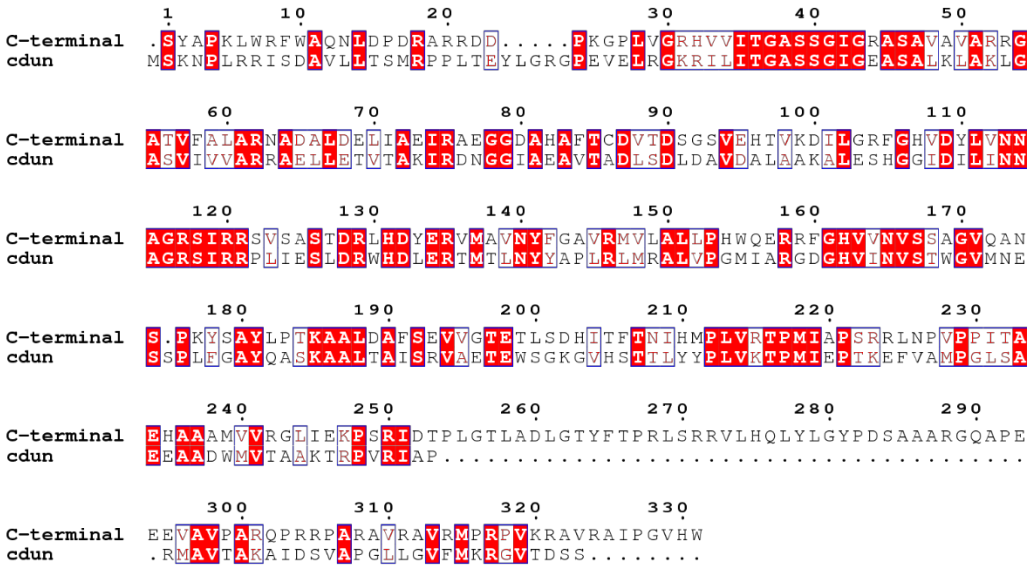

Figure S3 Amino acids sequence alignment between *Nb*Sal (*M. neoaurum*) and *Ps*Sal (*Pseudomonas sp.*).

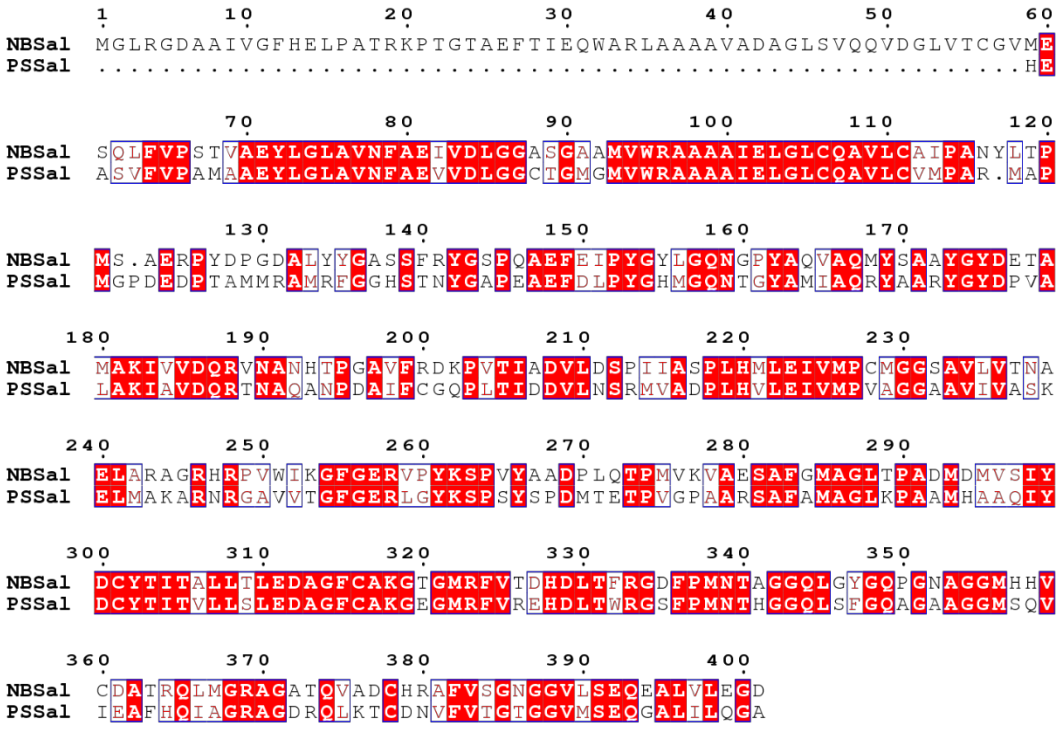

Figure S4 Optimization of biotransformation conditions for the improvement of ADD space-time yield by the engineered *M. neoaurum* NRRL B-3805$_{\Delta kshA1\text{-}opccR\text{-}sal\text{-}pL2\text{-}kstD1}$ under 30 g/L phytosterols. (A) Effect of different wet cell sediment. (B) Effects of different liquid volume. (C) The yields of ADD and AD under the conditions of 80g/L wet cell sediment and a liquid volume of 30 mL