

## SUPPLEMENTARY MATERIAL

**Table S1.** Primers used in this study

Primer name	Sequence (5'–3') <sup>a</sup>
AbLOX-F1	ATGCCTCGCCCTCAACAATTC
AbLOX-R1	CTCGCAATCTGAATCTGAGTCTACC
AbLOX-F	CATGCCATGGGC ATGCCTCGCCCTCAACAATTC
AbLOX-R	AAGGAAAAAAGCGGCCGCTCGCAATCTGAATCTGAG
AbLDS1-F1	ATGTCCCACTTGACAAATCTAC
AbLDS1-OL-R	CCCATAACTTCAATCGCCTTC
AbLDS1-OL-F	GAAGGCGATTGAAGTTATGGG
AbLDS1-R1	TTACACTTTCACCTGCAACTG
AbLDS1-F	<i>GGGTACCCTCGAGGGATCCGAATTCATGTCCCACTTGACAAATCTACTTCT</i>
AbLDS1-R	<i>TTTAAGCAGAGATTACCTATCTAGATTACACTTTCACCTGCAAC</i>
AbLDS2-F1	ATGTCCAAGAGGCTCTCTGG
AbLDS2-OL-R	CAGGATGGCAGACTAATGG
AbLDS2-OL-F	CCATTAGTCGTGCCATCCTG
AbLDS2-R1	AGCATCATACTGAATGGTAAGAGAC
AbLDS2-F	<i>TGGTGGTATCGAAGGTAGGCATATGATGTCCAAGAGGCTCTCTGGTATC</i>
AbLDS2-R	<i>TTTAAGCAGAGATTACCTATCTAGAAGCATCATACTGAATGG</i>
AbHPL-F1	ATGGTCCTTGACGTTACGCT
AbHPL-R1	TCATGCTTGCGCACGAGG
AbHPL-F	GGAATTCCATATGGTCCTTGACGTTACGCT
AbHPL-R	CCCGCGGCCGCTCATGCTTGCGCACGAGG
HRasAbLOX-F	<i>ACACAACAACCTTATCACTAGTCTCGCAATCTGAATCTGAGTCTACC</i>
HRasAbLOX-R	<i>TGCATGCCAATTCTAGAGGGCCCCCTCGCCCTCAACAATTCATT</i>

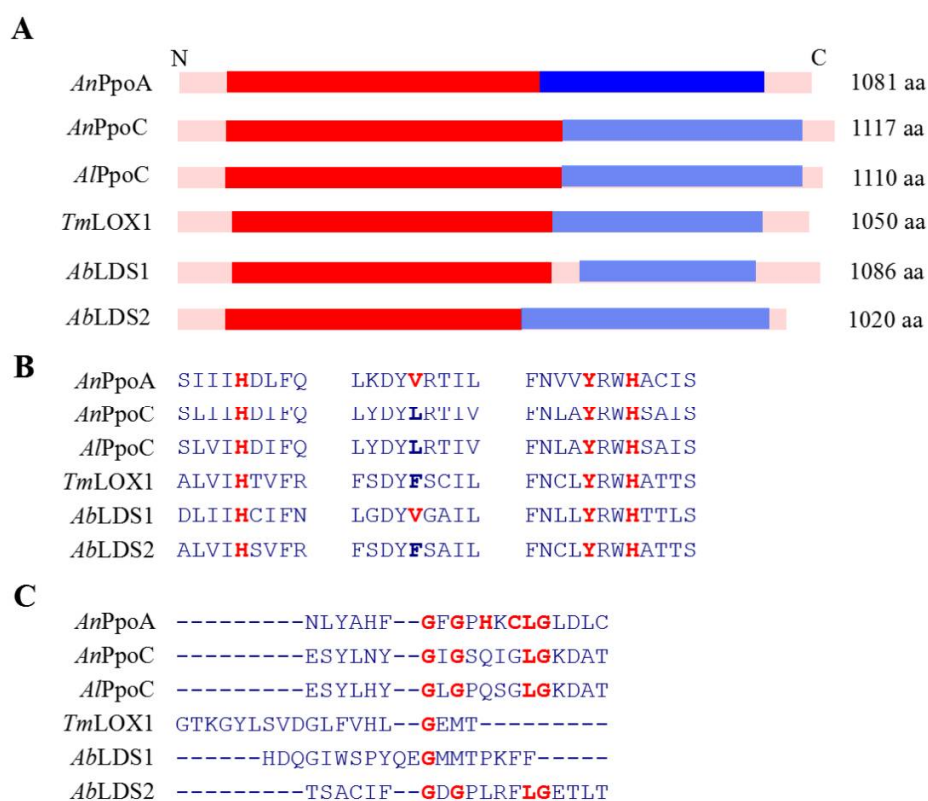
Hyg-F	CTTCTGCGGGCGATTTGTG
Hyg-R	TCGTTATGTTTATCGGCACTTT
asAbLOX-F	CTCGCAATCTGAATCTGAGTCTACC
asAbLOX-R	CCTCGCCCTCAACAATTCATT
Ef1a-F	AACAAGGTTCCCCTGGTGAC
Ef1a-R	CGGGTGATTGAGGACGATG
RT-LOX-F	AGCTCCGAGCGACTGTTGGC
RT-LOX-R	GGGTGCGTTTGATCTCGTCA

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<sup>a</sup> Italic letters show homology arm sequence

<i>AbLOX</i>	-MFRPQQFILPDLLSSCPLEDGLNPHYREAAAESRTWINSFNIFSNRKRAEFIQGLNEL	59
<i>Cop4</i>	MRPTARQFTLPDLFSICPLQDATNPWKQAAAESRAWINSYNIETTRKRAEFIQGSNEL	60
<i>AbLOX</i>	CSHVYCYAGYEEFRTTCDFVNVLFVVDEISDEQSGKDARATGLSYAESMRNADWDDNSVV	119
<i>Cop4</i>	CSHVYAYAGYEEFRTTCDFVNVLFVVDEISDDQNGQDARATGRIFVNAMRDAFWDDGSIL	120
<i>AbLOX</i>	AKITKEFRARLIRIRAGHNNFRFVASSDAYTRCVGREAELEAGEVLSLEEYIPLRRNNS	179
<i>Cop4</i>	AKITHEFRERFVRLAGPKTVRRFADLCESYTD CVAREAELELRERNOVLGLNDFIALRRONS	180
<i>AbLOX</i>	AVLLCFDLVEYILGVLDLPESIIYQNATFLKAYWAACDFVCWANDVYSYNVEQSKGHTGNNV	239
<i>Cop4</i>	AVLLCYSLVEYILGIDLDEVEYEDFTFAKAYWAACDFVCWANDVYSYDMEQAKGHTGNNV	240
<i>AbLOX</i>	VTVLMNDRQICLQEACDYIGDRCRQFMNDYLAARDELRA--TVGGDASRFIDALGYWIIIG	297
<i>Cop4</i>	VTVLMKEKDLSLQEASDYIGRECEKQMRDYLEAKSQLQSTDLPQFAVRYTEALGYWMVG	300
<i>AbLOX</i>	NMEWSFESPRYFGHEHDEIKRTLTTLTKPSEVHEE---VDSDSDE	340
<i>Cop4</i>	NLVWSFESQRYFGAHERVKATHVVHLRPSSVLEASCDSDSDSDC-	345

**Figure S1.** The amino acid sequence identify between *AbLOX* in *Agaricus bisporus* H97 and *Cop4* in *Coprinus cinereus*. *AbLOX*, putative lipoxygenase in *A. bisporus* H97 (NCBI accession number XP\_006459300); *Cop4*, linoleate 10R-lipoxygenase *Cop4* (NCBI accession number XP\_001836356).

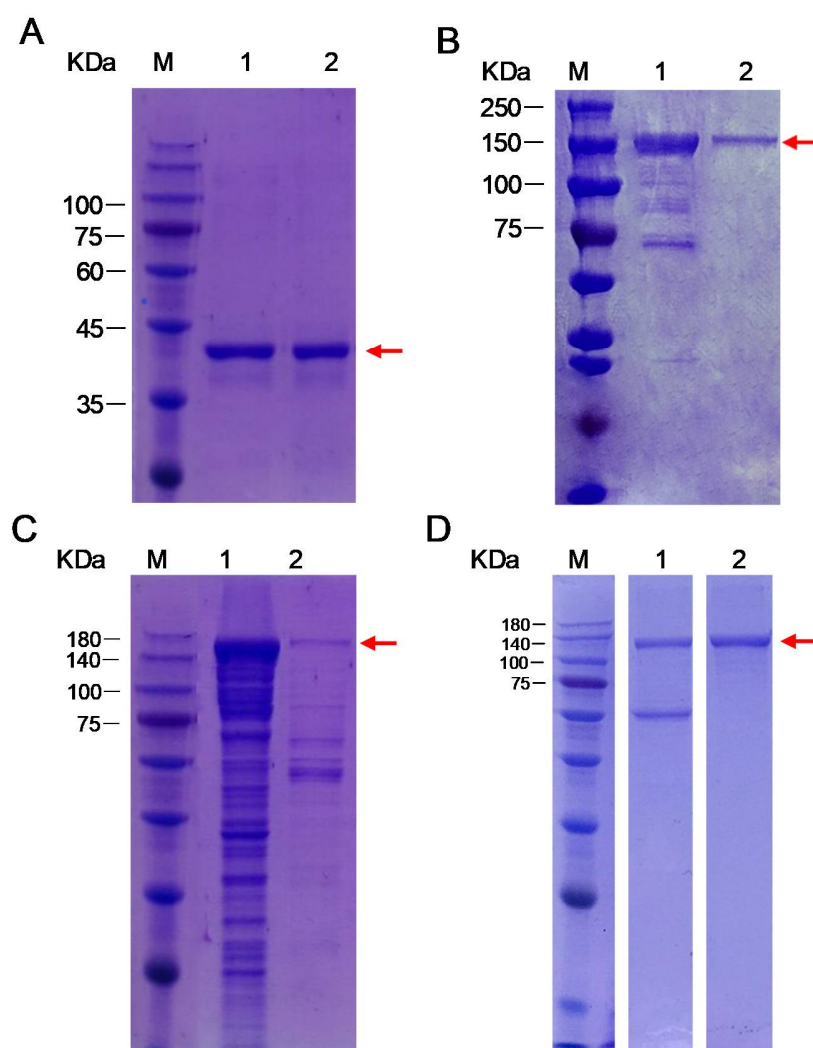


**Figure S2.** Domain structure and catalytic active site of the putative heme dioxygenase from *Agaricus bisporus* H97 and other selected fungi. A, Domain structure. Solid red box shows the heme peroxidase/dioxygenase domain; solid blue box shows the heme-thiolate domain with isomerization activity; solid light blue box shows the heme-thiolate domain without isomerization activity. B, Bold red letters show the conserved amino acid residues in the active site, bold purple letters show non-conserved amino acid residues in the active site. C, Heme-thiolate domain amino acid sequence alignment. Bold red letters show amino acid residues that are conserved in the catalytically active site. AbLDS1, linoleate diol synthase 1 in *A. bisporus* H97 (NCBI accession number XP\_006456120); AbLDS2, linoleate diol synthase 2 in *A. bisporus* H97 (NCBI accession number XP\_006461894); AnPpoA, *Aspergillus nidulans* PpoA

(NCBI accession number AAR88626); *AnPpoC*, *As. nidulans* PpoC (NCBI accession number AAT36614); *AlPpoC*, *As. luchuensis* PpoC (NCBI accession number GAT23542); *TmLOX1*, *Tricholoma matsutake* lipoxygenase 1 (NCBI accession number KAF8239383).

<i>TmHPL</i>	MSTKHSSLSAFIPSSSTSPPLYNFPVPSFLNITGKSF TWLYTTLAIVFALLVLEQSVYRY	60
<i>AbHPL</i>	MVL-----DVHAALNVLHNASYAP---AVQGSTLTWFTTFTGIIGGLLALQAVYRY	52
<i>TmHPL</i>	KKRHLPQAKWTTFITIGKFADSLSPITLEGYKKQWDGGLSAVSVFNIFIVMASSNDYARKI	120
<i>AbHPL</i>	KKRHLPQDSWTIPVIGRFADSMSPITMEGYKKQWDGGLSVSVFNIFIVMASSNQYARKI	112
<i>TmHPL</i>	LNSPGEFAEFCIVHAAKSILLSDNWVFLNGKAHTSYRRVLNSLFTRRALSTIYIPIEENITR	180
<i>AbHPL</i>	LNSPMYAEFALVHSGKLVIGSDNWVFLTGKDHVDYRKVLNLLFTRKALCIYLGIQDSVAR	172
<i>TmHPL</i>	KHEAKWLATASKESAPQTIMMTVRHLNMDTSLNVFCGKHISEEAALEINEKYWAITKALE	240
<i>AbHPL</i>	KHYTKWLDEAAADPSAKPTIMMTARQANMETSLEVFCCGYIPENTVEEINDKYWRITQSL	232
<i>TmHPL</i>	LVNFPFLALPGTKVYNATQARKSAIHWLEIAANRSKKAMANGAEPCMLDEWVQILNDPSY	300
<i>AbHPL</i>	LVNFPFLALPGTKVYKAIQARKFVMHWLEFAASKSKLAMAEGGTPECMDEWVSIMAEPCY	292
<i>TmHPL</i>	NGRRDFSDEEMAVLFSFLFASQDALSSAVIYGFQHLADHPEVLAKIREEQEKVRQGDYQ	360
<i>AbHPL</i>	KGRKDFSNYEMAVVFSFLFASQDAMSSCVIYGFQHLADHPDILEKVRQEQEVRMGNYE	352
<i>TmHPL</i>	KPLTLEMLDQMTYINAVVKESLRIKPPVTMIPYKALKAFPIISDDYVVPFGSMVIPSEFNS	420
<i>AbHPL</i>	DPLTLEMLDEMVLQAVVKESLRIKPPVTMVPYCKKPFPIINENYTVVFGSMVIPSEFNS	412
<i>TmHPL</i>	LHDPSVYPEPEITFNPDRLDPESANQNPKNFIFGSGPHRCIGFEYTFINIAIVLATAA	480
<i>AbHPL</i>	LHDSEIYPEPEKTHPERWLDSKGSANSNSQNYLVFGSGPHKCIGLEYAQMIIALMLANAA	472
<i>TmHPL</i>	VIMNIEHDVTPISDKVEIIATLFPKDGCRRLRSPRTQHP	519
<i>AbHPL</i>	VIMNFEHEITPRSGEVEIIATLFPKDGCRRLRSPRAQA-	510

**Figure S3.** The amino acid sequence identify between *AbHPL* in *Agaricus bisporus* H97 and *TmHPL* in *Tricholoma matsutake*. *AbHPL*, putative fatty acid hydroperoxide lyase in *A. bisporus* H97 (NCBI accession number XP\_006454582); *TmHPL*, fatty acid hydroperoxide lyase in *T. matsutake* (NCBI accession number KAF8221779).



**Figure S4.** Protein purification of the putative enzymes involved in 1-octen-3-ol biosynthesis by nickel ion affinity chromatography column. (A) *AbLOX*. Lane 1 and 2, elution with 100 and 150 mM imidazole. (B) *AbLDS1*. Lane 1 and 2, elution with 100 and 150 mM imidazole. (C) *AbLDS2*. Lane 1 and 2, elution with 20 and 100 mM imidazole. (D) *AbHPL*. Lane 1 and 2, elution with 100 and 150 mM imidazole.

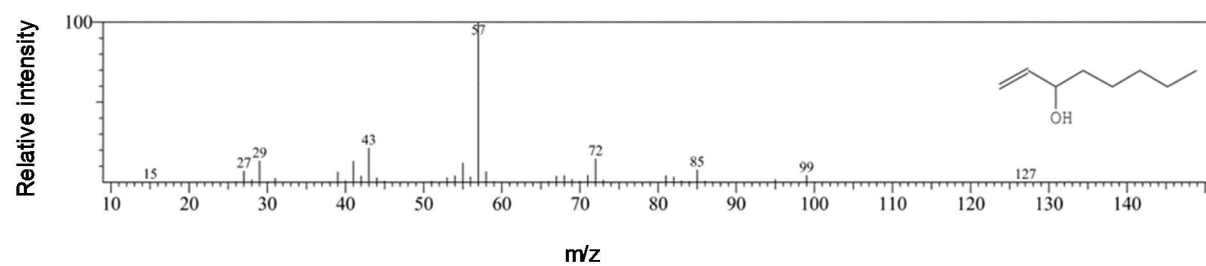


Figure S5 Mass spectrum of 1-octen-3-ol from the enzymatic linoleic acid reaction by *AbLOX*+*AbHPL*