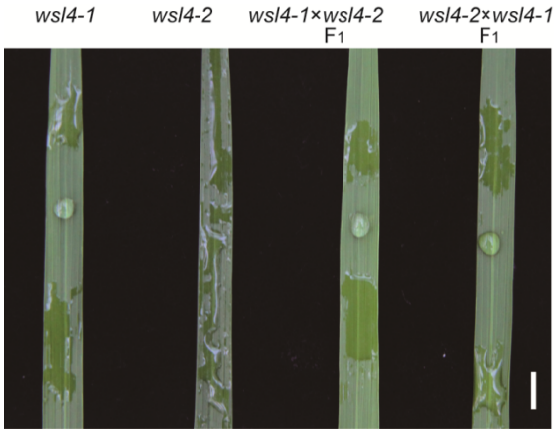


Supplemental Figure S1. Water retention of leaf blades in *wsl4-1*, *wsl4-2* and their F_1 generations. After reciprocal crosses, the leaf blades of F_1 generations from *wsl4-1* and *wsl4-2* showed sectored -wetting trait identical to *wsl4-1*. Scale bars = 1.0 cm.

MPGAAGYSGSVKLKYVKLGYQYLVNHFLTLLLVPVMAATALELARMGPGELLSLWRSLQL 60

DLVHILCSVFLVVFVGTVYFMSRPRPVYLVDYSCYKPPPSCRVPFATFMEHTRLITDDEK 120

SVRFQTRILERSGLGEETCLPPANHYIPPNPSMEASRAEAQLVIFSAIDDLVRRTGLKPK 180

DIDILVVNCSLFSPTPSLSAMIINKYKLRSNIRSFNLSGMGCSAGLISLDLARDMLQVHP 240

NSNALVVSTEIITPNFYWGTRRDMMLPNCLFRMGAAAILLSNRRREARRAKYRLMHVVRT 300

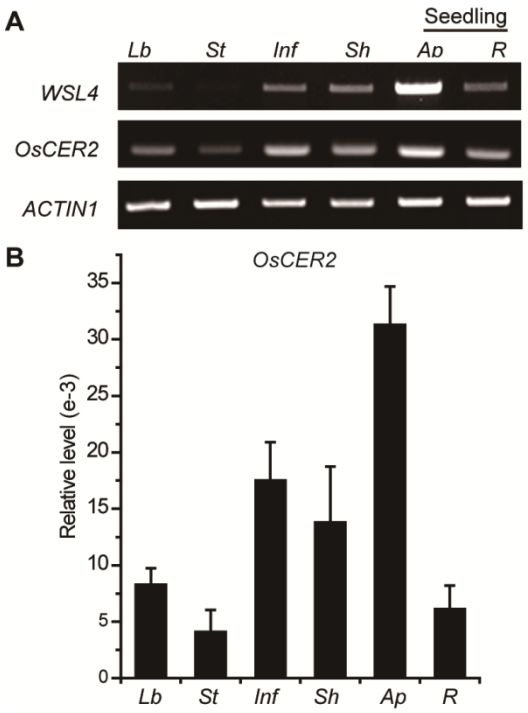
HKGADDRAYRCVYEEEDEQGHSGISLSKELMAIAGDALKSNITTIGPLVLPMSEQLLFFF 360

RLVGRKLINKKWKPYIPDFKLAFEHFCIHAGGRAVIDELQKNLDLSAQHVEASRMTLHRF 420

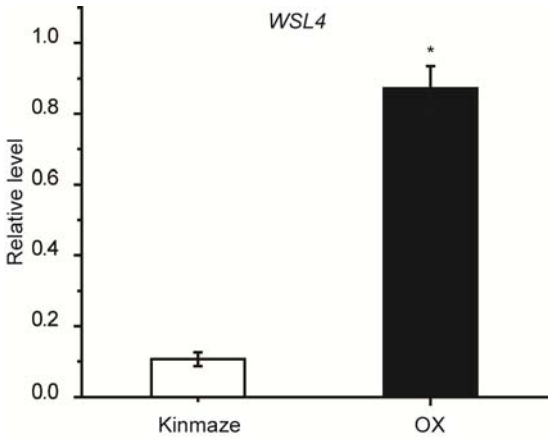
GNTSSSSLWYELAYIEAKGRMRRGDRVWQIGFGSGFKCNSAVWKCLRTVKTPADGPWDDC 480

IHRYPVDIPEVVKL 494

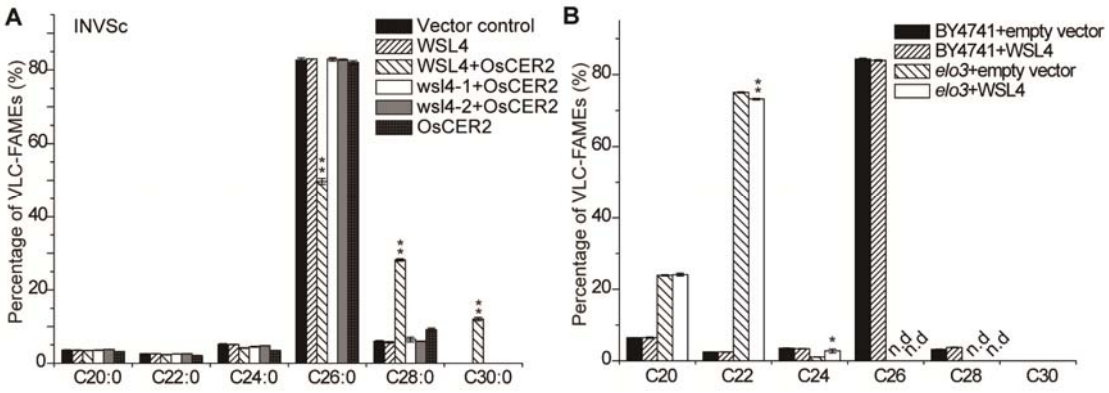
Supplemental Figure S2. The amino acid sequence of WSL4. The two predicted transmembrane domains are underlined; the mutations in *wsl4-1* and *wsl4-2* are indicated by black boxes; the predicted positions of active site residues are indicated by black arrows.



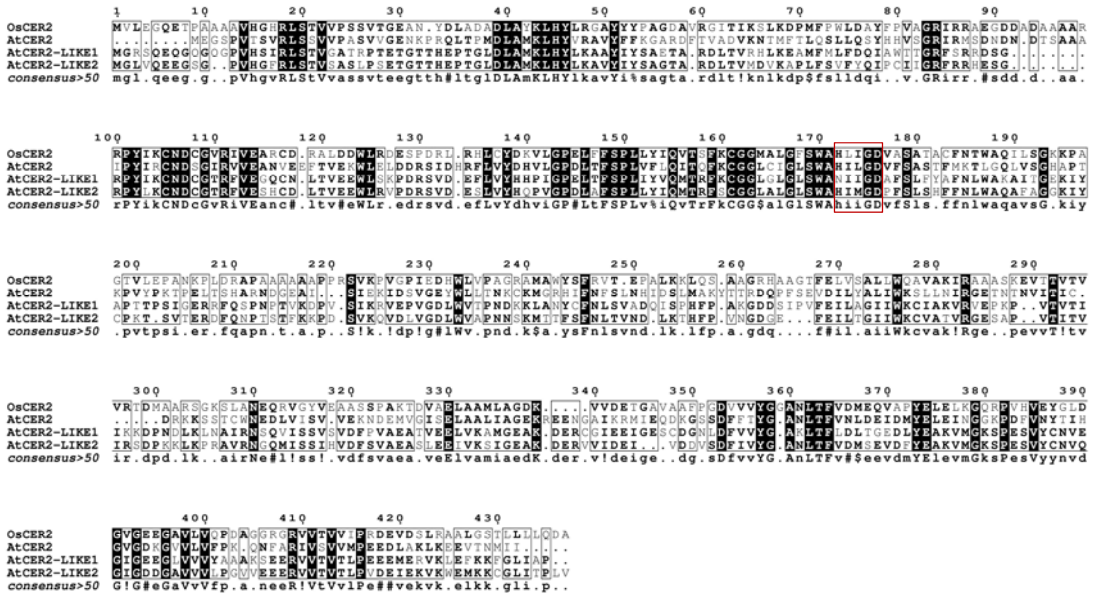
Supplemental Figure S3. Analysis of the expression pattern of *WLS4* and *OsCER2*. The cDNA was reverse transcribed from leaf blade (*Lb*), stem (*St*), inflorescence (*Inf*), sheath (*Sh*), aerial part (*Ap*) of two-week-old seedling and root of two-week-old seedling (*R*) for RT-PCR and qPCR. *Actin 1* gene was used as a control. All primers used are listed in the Supplemental Table S3.



Supplemental Figure S4. qPCR analysis of *WSL4* expression in the overexpression line. Data are given as mean ± SE (n = 3). The asterisk indicates significant difference ($P < 0.05$) determined in a Student's *t* test.

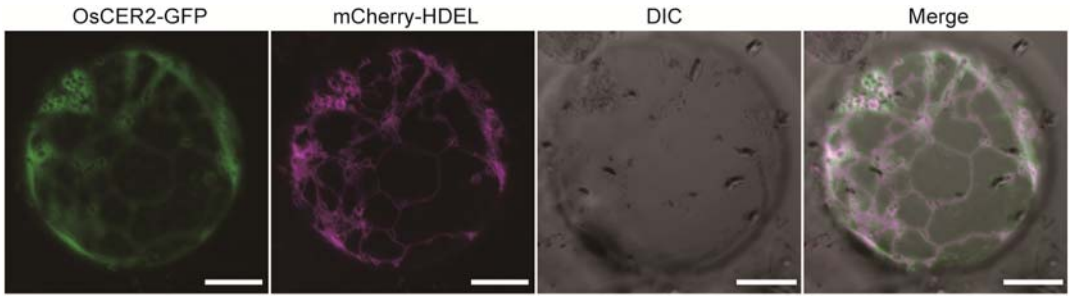


Supplemental Figure S5. Profiles of VLCFAs in yeast cells expressing *WSL4* and *OsCER2*. Expressing the empty vector, *WSL4*, *OsCER2*, *WSL4* & *OsCER2*, *wsl4-1* & *OsCER2*, and *wsl4-2* & *OsCER2* in wild-type INVSc yeast (A) and expressing *WSL4* alone in wild-type BY4741 and *elo3* yeast mutant (B). Data are mean ± SE of three biological replicates. *, $P < 0.05$; **, $P < 0.01$. *n*-nonadecanoic acid was used as the internal standard.

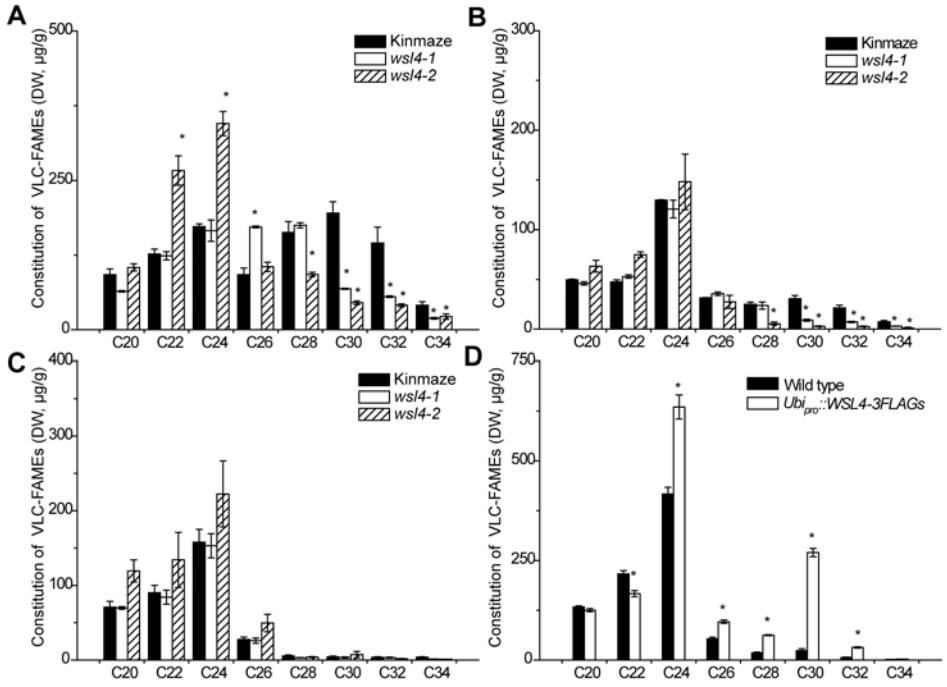


Supplemental Figure S6. Alignment of the amino acid sequences of OsCER2 with AtCER2s. The amino acid sequences of OsCER2 (Os04g0611200) were aligned with that of AtCER2 (At4g24510), AtCER2-LIKE1 (At4g13840), and AtCER2-LIKE2 (At3g23840) by Hierarchical Clustering Analysis as described by Corpet (1988). The conserved HxxxD motif is boxed. Identical amino acids are shown on a black background, and gaps are indicated by dots.

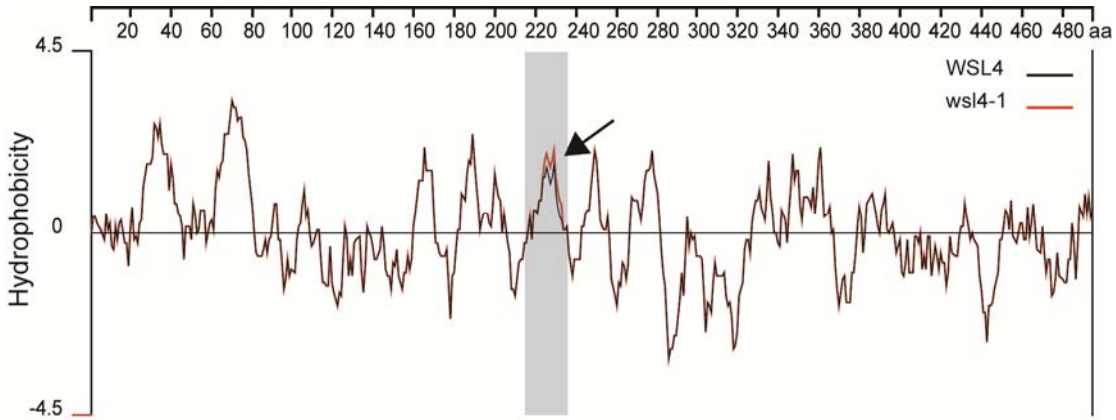
Corpet F (1988) Multiple sequence alignment with hierarchical clustering. Nucl Acids Res **16:** 10881-10890



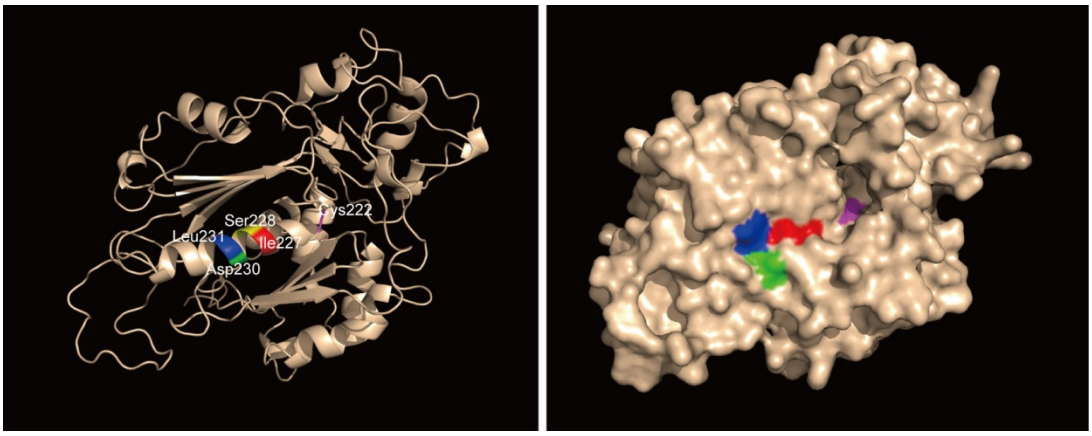
Supplemental Figure S7. Subcellular location of OsCER2. Co-expression of OsCER2-GFP fusion protein and the mCherry-HDEL fusion protein in the rice protoplasts. Scale bars = 10 μm.



Supplemental Figure S8. Profiles of fatty acids distribution in rice organs and tissues. GC-MS analysis of VLCFAs from leaf blades with (A) and without (B) epicuticular wax, stem at booting stage (C) and rice calli (D). Data are expressed as means ± S.E of three biological replicates. *, $P < 0.05$; **, $P < 0.01$.



Supplemental Figure S9. Hydrophilicity plot (Kyte-Doolittle) for WSL4 and wsl4-1 peptides. The analyses were performed using Protean, Lasergene Biocomputing Software with Residue to Average set to default value 9. Arrow indicates the enhanced-Hydorphilicity region in the wsl4-1 peptide compared with that in the WSL4 peptide.



Supplemental Figure S10. Three-dimensional homology modeling of WSL4. The sequence of WSL4 was used to search and select suitable templates for modeling at the Swiss Model website (http://swissmodel.expasy.org//SWISS-MODEL.html). A three-dimensional homology model of WSL4 was generated using 1qlv.1.A, 1qlv.1.A, and 1eeo.1.A as the templates. Cys222, Ile227, Ser228, Asp230, and Leu231 residues are colored by magenta, red, bright yellow, green, and blue, respectively.

Supplemental Table S1. Genotype for 14 markers from chromosome 3 across eight recombinants of F_3 offspring originating from a *wsl4-1* (*japonica*) × 9311 (*indica*) cross. SSR positions are indicated as described in McCouch et al. (2002). Mapping data format 'AHB' with A = homozygous wsl4-1, H = heterozygous and B = homozygous '9311', Chr, chromosome, IND, individual of recombinants.

SSR name	RM14511	RM6038	RM14530	RM7576	RM14602	C1	C4	C5	C7	C8	**C9**	**RM14612**	RM14614	RM14635
Position [Mbp]	4.661520	4.811234	4.114305	6.046794	6.134606	6.156255	6.157962	6.167017	6.218263	6.235762	**6.239004**	**6.502880**	6.521256	6.820547
Chr.	3	3	3	3	3	3	3	3	3	3	**3**	**3**	3	3
wsl4-1	A	A	A	A	A	A	A	A	A	A	**A**	**A**	A	A
9311	B	B	B	B	B	B	B	B	B	B	**B**	**B**	B	B
IND-12-4	B	H	H	H	H	H	H	H	H	H	**H**	**H**	H	H
IND-11-1	H	H	H	A	A	A	A	A	A	A	**A**	**A**	A	A
IND-26-3	A	A	A	A	A	A	A	A	A	A	**A**	**H**	H	H
IND-26-6	A	A	A	A	A	A	A	A	A	A	**A**	**H**	H	H
IND-13-14	A	A	A	A	A	A	A	A	A	A	**A**	**A**	A	H
IND-13-18	H	H	H	H	H	H	H	H	H	H	**H**	**A**	A	A
IND-13-20	B	B	B	B	B	B	B	B	B	B	**B**	**B**	B	B
IND-13-21	A	A	A	A	A	A	A	A	A	A	**A**	**H**	H	H

Supplemental Table S2. Mass-spectrometry identification of OsCER2 peptides.

Contrib	Conf	Sequence	Cleavages	dMass	Prec MW	Prec m/z	Theor MW	Theor m/z
2	99.00000095	AALGSTLLLLQDA		-0.000959366	-0.000959366	643.3713	1284.729	643.3718
2	99.00000095	AEGDDADAAAAR		-0.00146701	-0.00146701	566.7462	1131.479	566.7469
2	99.00000095	ALDDWLRDESPDR	missed R-D@7	0.00193802	0.00193802	529.9188	1586.733	529.9181
2	99.00000095	APAAAAAAPPR		-0.00169661	-0.00169661	482.2713	962.5298	482.2722
2	99.00000095	EVTTVTVVR		0.000204001	0.000204001	502.2928	1002.571	502.2928
2	99.00000095	GAYYYPAGDAVR		-0.0005297	-0.0005297	651.809	1301.604	651.8093
2	99.00000095	GGANLTFVDMEQVAPYELELK	cleaved Y-G@N-term	0.00225488	0.00225488	775.3882	2323.141	775.3875
2	99.00000095	KPAGTVLEPANKPLDR		0.000997718	0.000997718	427.2456	1704.952	427.2453
2	99.00000095	LSTVVPSSVTGEANYDLADADLAYK		-0.031072499	-0.031072499	664.0547	2652.221	664.0625
2	99.00000095	LTFVDMEQVAPYELELK	cleaved N-L@N-term	0.00165015	0.00165015	675.6804	2024.018	675.6798
2	99.00000095	PAAAAVHGHR	cleaved T-P@N-term	-0.0029717	-0.0029717	493.7661	985.5206	493.7676
2	99.00000095	PAGTVLEPANKPLDR	cleaved K-P@N-term	-0.00174649	-0.00174649	526.6258	1576.857	526.6264
2	99.00000095	PVGPIEDHWLVPAGR	cleaved K-P@N-term	-0.000699272	-0.000699272	548.2946	1641.863	548.2949
2	99.00000095	PVHVEYGLDGVGEEGAVLVQPDAGGR	cleaved R-P@N-term	-0.00044266	-0.00044266	874.1047	2619.293	874.1049
2	99.00000095	RAEGDDADAAAAR	missed R-A@1	0.000443195	0.000443195	430.2009	1287.58	430.2007

Fifteen peptides of OsCER2 (accession number: Os04g0611200) were identified by LC-MS; the coverage rate reached 56.29%.

Supplemental Table S3. Primers.

Reaction	Primer name	Sequence information
Genomic fragment cloning	WSL4RE-F1	5'-ATTCACGAAACCACCTGCCAC
	WSL4RE-R1	5'-AGACAAGTTCAGGCAGAGCAGTG
	WSL4RE-F2	5'-ACCAACCTACTATCGGATGTGAC
	WSL4RE-R2	5'-GGCTTGTAGCAGGAGTAGTCG
	WSL4RE-F3	5'-CGGGGAAGTAGTGAGAGCATC
	WSL4RE-R3	5'-GAGGTGTAGGGGAGGTAATGC
	WSL4RE-F4	5'-TCAACAAGAAGTGGAAGCCG
	WSL4RE-R4	5'-AGATCGGGCATGTAAGTAAACC
Over-expression and	WSL4 cDNA-F1	5'-AGAGCATCGATCATGCCG
subcellular localization	WSL4 cDNA-R1	5'-CGATCACAGCTTGACGACC
	OsCER2 cDNA-F1	5'-AAGCGCGATTCCCTTGCCATG
	OsCER2 cDNA-R1	5'-TCAGGCGTCTTGCAGCAGCAG
Promoter GUS assay	WSL4GUS-F1	5'-ACGCgtcgacTACAATCCTGTCCCACGAAG
	WSL4GUS-R1	5'-TCCcccgggGATCGATGCTCTCACTACTTCC
Fatty acid analysis	AtCER2YE-F1	5'-AATTGAATTCATGGAGGGAAGCCCAGTGAC
	AtCER2YE-R1	5'-AATTGCGGCCGCTATAATCATATTAGTCACCTCCTCC
	WSL4YEOP-F1	5'-ggatccTTAATAATGCCAGGTGCTGCTGGTT
	WSL4YEOP-R1	5'-gaattcTTTACAACTTGACAACTTCTGG
	OsCER2YE-F1	5'-CCGgaattcATGGTGCTCGAGGGGCAGG
	OsCER2YE-R1	5'-GGACTAGTTCAGGCGTCTTGCAGCAGCAG
Gene expression analysis	WSL4REAL-F1	5'-ACCACTACATCCCGCCTAAC
	WSL4REAL-R1	5'-AGACTGCAGTTGACGACGAG
	OsCER21rt-F1	5'-CTCTGCTACGACAAGGTGCTC
	OsCER21rt-R1	5'-TGACGCGGAAGGAGTACCAC
	OsCER21REAL-F1	5'-CATGGCGTGGTACTCCTTC
	OsCER21REAL-R1	5'-GAGACGAGCTCGAACGTG
Yeast-two-hybrid assay	WSL4Y2H-F1	5'- TACTCTTATggccattacggccATGCCAGGTGCTGCTGGTT
	WSL4Y2H-R1	5'-TACTCTTATggccgaggcggccGCCAACTTGACAACTTCTG
	OsCER2Y2H-F1	5'-TACTCTTATggccattacggccATGGTGCTCGAGGGGCAGG
	OsCER2Y2H-R1	5'-TACTCTTATggccgaggcggccGCGGCGTCTTGCAGCAGCAGG
Site-directed mutagenesis	WSL4MuS_{228}F-F	5'-TTCTGCTGGTTTGATTTTTTTAGATTTGGC
	WSL4MuS_{228}F-R	5'-AAAATCAAACCAGCAGAACAACCCATACCAG
	WSL4MuN_{422}S-F	5'-CCTTGCATAGATTCGGTAGTACCTCCAGTTC
	WSL4MuN_{422}S-R	5'-CTACCGAATCTATGCAAGGTCATTCTACTAG
	WSL4c#-F1	5'-CCAGACCAGGATTCTTGAGCGG
	WSL4c#-R1	5'-AACCGCACGCTCTTCTCGTCGTC
	WSL4c#-F2	5'-GTTCCAGACCAGGATTCTTGAGCGGTCGGG
	WSL4c#-R2	5'-AAGAATCCTGGTCTGGAACCGCACGCTCTTC
	WSL4c#-F3	5'-GAAGTACGTGAAGCTGGGTTAC
	WSL4c#-R3	5'-AGCTTCACCGACCCGGAGTAC
	OsCER2H_{172}A-F	5'-CAGCTGGGCGGCCCTCATCGGC
	OsCER2H_{172}N-F	5'-CAGCTGGGCGAACCTCATCGGC

	$OsCER2H_{172}E$-F	5’-CAGCTGGGCGGAGCTCATCGGC
	$OsCER2H_{172}MU$-R	5’-AAGCCGAGCGCCATGCCACCGC
	$OsCER2D_{176}A$-F	5’-GCACCTCATCGGCGCCGTGGCG
	$OsCER2D_{176}H$-F	5’-GCACCTCATCGGCCACGTGGCG
	$OsCER2D_{176}MU$-R	5’-GCCCAGCTGAAGCCGAGCGCC
InDel marker	C9-F	5’-TCTAGTTCGGCTAGTCGGC
	C9-R	5’-GTCTCGCAAAGTAGTCGAAATC

Supplemental Table S4. Transgenic yeast cell lines.

Genes expressed	Expression vectors		Selection medium
WSL4	pYES2-*WSL4*	pESCHis	-Ura/-His
OsCER2	pYES2	pESCHis-*OsCER2*	-Ura/-His
AtCER2	pYES2	pESCHis-*OsCER2*	-Ura/-His
WSL4+OsCER2	pYES2-*WSL4*	pESCHis-*OsCER2*	-Ura/-His
WSL4+AtCER2	pYES2-*WSL4*	pESCHis-*OsCER2*	-Ura/-His
wsl4-1+OsCER2	pYES2- *wsl4-1*	pESCHis-*OsCER2*	-Ura/-His
wsl4-2+OsCER2	pYES2- *wsl4-2*	pESCHis-*OsCER2*	-Ura/-His
WSL4+$OsCER2_{H172N}$	pYES2-*WSL4*	pESCHis-$OsCER2_{H172N}$	-Ura/-His
WSL4+$OsCER2_{H172E}$	pYES2-*WSL4*	pESCHis-$OsCER2_{H172E}$	-Ura/-His
WSL4+$OsCER2_{D176A}$	pYES2-*WSL4*	pESCHis-$OsCER2_{D176A}$	-Ura/-His
WSL4+$OsCER2_{D176H}$	pYES2-*WSL4*	pESCHis-$OsCER2_{D176H}$	-Ura/-His