

Supplementary Information

Evolutionary alterations in gene expression and enzymatic activities of gibberellin 3-oxidase 1 in *Oryza*

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Supplementary Tables

Data collection	
Beam line	SPring-8 BL26B1
Wavelength (Å)	1.0
Detector	MX225HE
Space group	P21
Molecules (Asymmetric Unit)	1
Cell dimensions (Å, °)	$a = 56.84, b = 45.59, c = 68.54$ $\alpha = 90.00, \beta = 102.25, \gamma = 90.00$
Resolution (Å) *	50.00–1.9 (1.93–1.9)
Total reflections	117157
Unique reflections	27238
Completeness (%) *	99.7 (95.7)
R_{merge} (%) *	4.7 (22.1)
Average $\langle I \rangle / \langle \sigma(I) \rangle$ *	38.4 (7.8)
Refinement statistics	
Number of residues	354
Number of water molecules	296
$R_{\text{working}}/R_{\text{free}}$ (%)	16.51/20.82
R.m.s.d.bond length (Å)	0.010
R.m.s.d.bond angles (deg)	1.307

* Data for the highest resolution shell are listed in parentheses.

Supplementary Table 1. Data collection and refinement statistics¹⁻⁷ of OsGA3ox2.

Plant species	Gene name in this study	Gene ID	Data Source*
<i>Oryza sativa</i>	<i>Oryza_sativa_GA3ox1, OsGA3ox1</i>	LOC_Os05g08540	TIGR
	<i>Oryza_sativa_GA3ox2, OsGA3ox2</i>	LOC_Os01g08220	
<i>Oryza nivara</i>	<i>Oryza_nivara_GA3ox1</i>	ONIVA05G05590	EnsemblPlants
	<i>Oryza_nivara_GA3ox2</i>	ONIVA01G05670	
<i>Oryza rufipogon</i>	<i>Oryza_rufipogon_GA3ox1</i>	ORUFI05G05470	EnsemblPlants
	<i>Oryza_rufipogon_GA3ox2</i>	ORUFI01G05300	
<i>Oryza barthii</i>	<i>Oryza_barthii_GA3ox1</i>	OBART05G05200	EnsemblPlants
	<i>Oryza_barthii_GA3ox2</i>	Not annotated	
<i>Oryza meridionalis</i>	<i>Oryza_meridionalis_GA3ox1</i>	OMERI07G10700	EnsemblPlants
	<i>Oryza_meridionalis_GA3ox2</i>	OMERI01G05010	
<i>Oryza glumipatula</i>	<i>Oryza_glumipatula_GA3ox1</i>	OGLUM05G05060	EnsemblPlants
	<i>Oryza_glumipatula_GA3ox2</i>	OGLUM01G05460	
<i>Oryza punctata</i>	<i>Oryza_punctata_GA3ox1</i>	OPUNC05G04790	EnsemblPlants
	<i>Oryza_punctata_GA3ox2</i>	OPUNC01G04850	
<i>Oryza brachyantha</i> (W0654)	<i>Oryza_brachyantha_GA3ox1</i>		This study
	<i>Oryza_brachyantha_GA3ox2</i>		
<i>Oryza granulata</i> (W0008)	<i>Oryza_granulata_GA3ox1</i>		This study
	<i>Oryza_granulata_GA3ox2</i>		
<i>Leersia perrieri</i> (W1529)	<i>Leersia_perrieri_GA3ox1</i>	LPERR05G4620	EnsemblPlants
	<i>Leersia_perrieri_GA3ox2</i>	Not annotated	
<i>Hordeum vulgare</i>	<i>Hordeum_vulgare_GA3ox1</i>	AY551430	NCBI
	<i>Hordeum_vulgare_GA3ox2</i>	AY551431	
<i>Triticum aestivum</i>	<i>Triticum_aestivum_GA3ox-2A</i>	TraesCS2A02G540400	EnsemblPlants
	<i>Triticum_aestivum_GA3ox-2B</i>	TraesCS2B02G570900	
	<i>Triticum_aestivum_GA3ox-2D</i>	TraesCS2D02G542100	
	<i>Triticum_aestivum_GA3ox-3A</i>	TraesCS3A02G122600	EnsemblPlants
	<i>Triticum_aestivum_GA3ox-3B</i>	TraesCS3B02G141800	
	<i>Triticum_aestivum_GA3ox-3D</i>	TraesCS3D02G124500	
<i>Brachypodium distachyon</i>	<i>Brachypodium_distachyon_GA3ox2a</i>	Bradi2g04840(a)	EnsemblPlants
	<i>Brachypodium_distachyon_GA3ox2b</i>	Bradi4g23570(b)	
<i>Oropetium thomacum</i>	<i>Oropetium_thomacum_Oropetium_20150105_07284A</i>	Oropetium_20150105_07284A	phytozome
<i>Sorghum bicolor</i>	<i>Sorghum_bicolor_GA3ox1</i>	Sobic.009G064700	phytozome
	<i>Sorghum_bicolor_GA3ox2</i>	Sobic.003G045900	
<i>Zea mays</i>	<i>Zea_mays_GA3ox1</i>	Zm00001d037627_T001	EnsemblPlants
	<i>Zea_mays_GA3ox2</i>	Zm00001d039634_T001	
<i>Setaria italica</i>	<i>Setaria_italica_Si025127m.g</i>	Si025127m.g	EnsemblPlants
	<i>Setaria_italica_Si001832m.g</i>	Si001832m.g	
<i>Panicum virgatum</i>	<i>Panicum_virgatum_Pavir.J24392.1</i>	Pavir.J24392.1	phytozome
	<i>Panicum_virgatum_Pavir.Eb00358.1</i>	Pavir.Eb00358.1	
	<i>Panicum_virgatum_Pavir.J17485.1</i>	Pavir.J17485.1	
<i>Ananas comosus</i>	<i>Ananas_comosus_Aco009790.1</i>	Aco009790.1	phytozome
	<i>Ananas_comosus_Aco014326.1</i>	Aco014326.1	
<i>Musa acuminata</i>	<i>Musa_acuminata_GA3ox1</i>	GSMUA_Achr1T03100_001	EnsemblPlants
	<i>Musa_acuminata_GA3ox2</i>	GSMUA_Achr4T08970_001	
	<i>Musa_acuminata_GA3ox3</i>	GSMUA_Achr5T09790_001	
	<i>Musa_acuminata_GA3ox4</i>	GSMUA_Achr7T13240_001	
	<i>Musa_acuminata_GA3ox5</i>	GSMUA_AchrUn_randomT03870_001	
<i>Spirodela</i>	<i>Spirodela_polyrhiza_Spiro21G0010100</i>	Spiro21G0010100 (primary)	phytozome

<i>polyrhiza</i>	<i>Spirodela polyrhiza_Spipo21G0010200</i>	Spipo21G0010200 (primary)	
<i>Zostera marina</i>	<i>Zostera_marina_Zosma208g00280.1</i>	Zosma208g00280.1	phytozome
<i>Arabidopsis thaliana</i>	<i>Arabidopsis_thaliana_GA3ox1</i>	AT1G15550	TAIR
	<i>Arabidopsis_thaliana_GA3ox2</i>	AT1G80340	
	<i>Arabidopsis_thaliana_GA3ox3</i>	AT4G21690	
	<i>Arabidopsis_thaliana_GA3ox4</i>	AT1G80330	
<i>Glycine max</i>	<i>Glycine_max_Glyma.04G071000</i>	Glyma.04G071000	phytozome
	<i>Glycine_max_Glyma.06G072600</i>	Glyma.06G072600	
	<i>Glycine_max_Glyma.07G033800</i>	Glyma.07G033800	
	<i>Glycine_max_Glyma.08G208300</i>	Glyma.08G208300	
	<i>Glycine_max_Glyma.13G361700</i>	Glyma.13G361700	
	<i>Glycine_max_Glyma.14G128400</i>	Glyma.14G128400	
	<i>Glycine_max_Glyma.15G012100</i>	Glyma.15G012100	
	<i>Glycine_max_Glyma.17G205300</i>	Glyma.17G205300	
<i>Solanum lycopersicum</i>	<i>Solanum_lycopersicum_GA3ox1</i>	Solyc06g066820	phytozome
	<i>Solanum_lycopersicum_GA3ox2</i>	Solyc03g119910	
	<i>Solanum_lycopersicum_GA3ox3</i>	Solyc01g058250	
	<i>Solanum_lycopersicum_GA3ox-like-1</i>	Solyc00g007180	
	<i>Solanum_lycopersicum_GA3ox-like-2</i>	Solyc05g052740	
<i>Vitis vinifera</i>	<i>Vitis_vinifera_GA3ox1</i>	GSVIVT01008811001	phytozome
	<i>Vitis_vinifera_GA3ox2</i>	GSVIVT01017173001	
	<i>Vitis_vinifera_GA3ox3</i>	GSVIVT01017178001	
	<i>Vitis_vinifera_GA3ox4</i>	GSVIVT01020680001	
	<i>Vitis_vinifera_GA3ox5</i>	GSVIVT01026928001	
	<i>Vitis_vinifera_GA3ox6</i>	GSVIVT01035796001	
<i>Amborella trichopoda</i>	<i>Amborella_trichopoda_evm_27.model.AmTr_v1.0_scaffold00122.35</i>	evm_27.model.AmTr_v1.0_scaffold00122.35 (primary)	phytozome
<i>Pinus tabuliformis</i>	<i>Pinus_tabuliformis_AHW42463</i>	AHW42463	NCBI
	<i>Pinus_tabuliformis_AHW42464</i>	AHW42464	
<i>Picea abies</i>	<i>Picea_abies_MA_10249g0010</i>	MA_10249g0010	ConGenIE
	<i>Picea_abies_MA_24155g0010</i>	MA_24155g0010	
<i>Lygodium japonicum</i>	<i>Lygodium_japonicum_Isotig12104</i>	Isotig12104	Lygodium japonicum Transcriptome Database
	<i>Lygodium_japonicum_Isotig09392</i>	Isotig09392	
<i>Selaginella moellendorffii</i>	<i>Selaginella_moellendorffii_GA3ox</i>	446928 (primary)	phytozome
<i>Physcomitrella patens</i>	<i>Physcomitrella_patens_GA3oxL</i>	Pp3c17_7090V3.1 (primary)	phytozome
<i>Oryza sativa</i>	<i>Oryza_sativa_GA20ox2, OsGA20ox2</i>	LOC_Os01g66100	TIGR
	<i>OsGA20ox1</i>	LOC_Os03g63970.1	
	<i>OsGA20ox3</i>	LOC_Os07g07420.1	
	<i>OsGA20ox4</i>	LOC_Os05g34854.1	
	<i>OsGA2ox1</i>	LOC_Os05g06670.1	
	<i>OsGA2ox2</i>	LOC_Os01g22910	
	<i>OsGA2ox3</i>	LOC_Os01g55240	
	<i>OsGA2ox4</i>	LOC_Os05g43880	
	<i>OsGA2ox5</i>	LOC_Os07g01340	
	<i>OsGA2ox6</i>	LOC_Os04g44150	
	<i>OsGA2ox7</i>	LOC_Os01g11150	
	<i>OsGA2ox8</i>	LOC_Os05g48700	
	<i>OsGA2ox9</i>	LOC_Os02g41954.1	
	<i>OsGA2ox10</i>	LOC_Os05g11810.1	

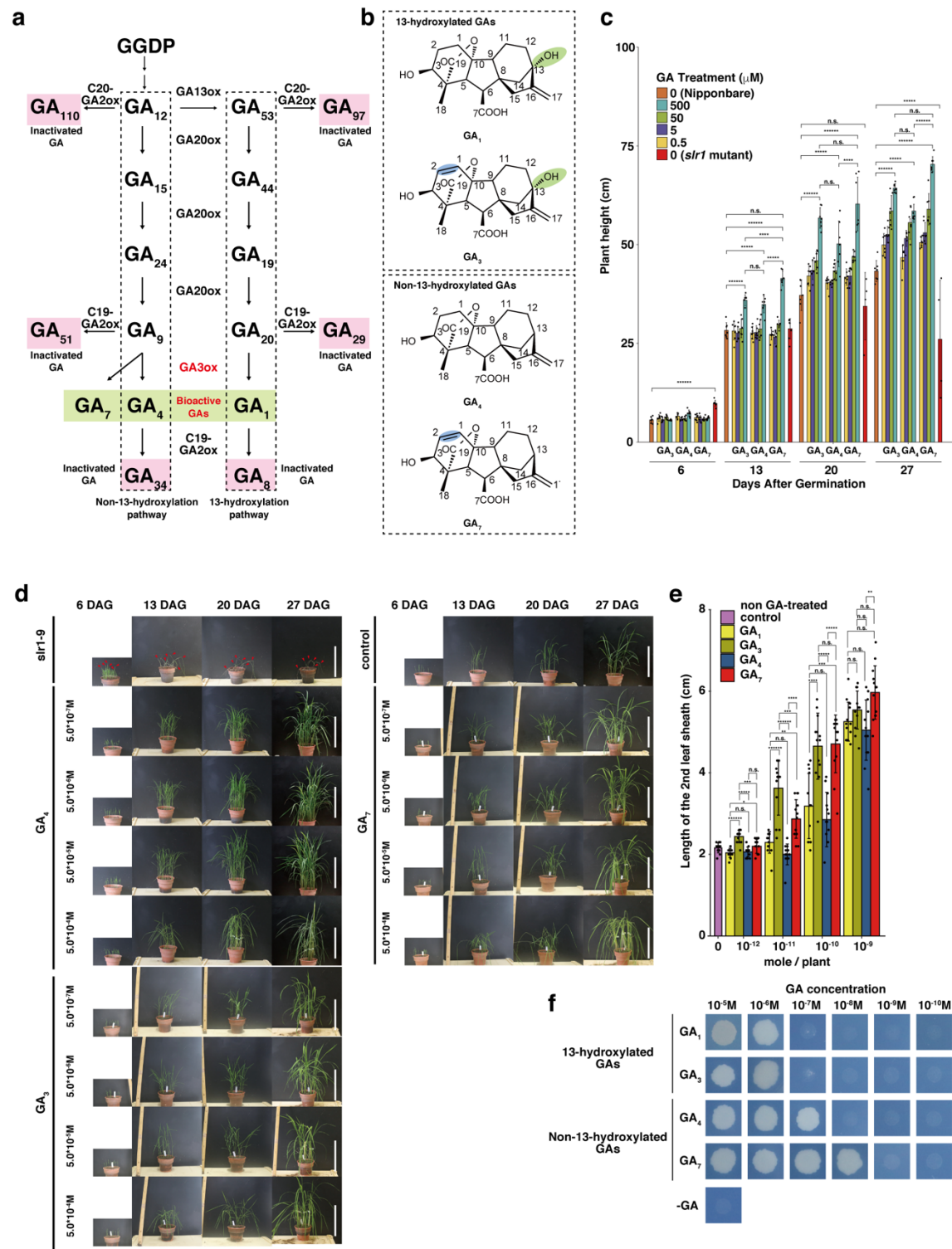
*TIGR, The Institute for Genomic Research Rice Genome Annotation project (<http://rice.plantbiology.msu.edu/>); EnsemblPlants (<http://plants.ensembl.org/index.html>); Phytozome (<https://phytozome.jgi.doe.gov/pz/portal.html>); NCBI (<https://blast.ncbi.nlm.nih.gov/Blast.cgi>); Lygodium japonicum Transcriptome Database (<http://bioinf.mind.meiji.ac.jp/kanikusa/>); ConGenIE (<http://congenie.org>); TAIR The Arabidopsis Information Resource (<http://www.arabidopsis.org/index.jsp>).

Supplementary Table 2. GA3ox orthologs of land plants used in this study.

Experiments	Sequence (5'-3')	Description
Cas9 target	GTTGACATCTGCTTCGGGTACCGG AAACCCGGTACCCGAAGCAGATGT	Cas9 target (guide RNA)
Genotyping	ATCACGCCGAAAGAAATGGTC	For <i>OsGA3ox1</i>
	ATGATGGGTGCAGTGCTATG	
	AACTCACGACAAAAATGCTT	For <i>O.brachyanthaGA3ox1</i>
	TGCAGGATGAAGGTGAAGAA	
	ATCTCGCGGCAAAAACGCTC	For <i>O.granulataGA3ox1</i>
	GGTGTGTCGACGTCACGTGAG	
	CCCGCAAGTCTGAAGAACT	For Cas9
GUS transgenic	ATACCTGGGCCTTTCTGGAT	
	cctctagaCGTACACACCTGACAACAAC	For <i>GUS</i>
	CCcccgggCTTGAAGAACATGGCGAGG	
	GCTTCTGCTGCTACGAGACC	For <i>GA3ox1</i> gene cloning of <i>Oryza</i>
	AGGTCCTCTTCTCCGAGCAC	<i>GA3ox1s</i> and <i>L.perrieriGA3ox1</i>
5'/3' RACE	TATTGGGTACTACTAAAGCTTAACGAATTCGCCCTT	For In-fusion
	GGGAAATTCGAGCTCGGTACCCGCGAATTCGCCCTT	
	CATGTGGTCCGAGGGCTACACC	For sequencing of <i>Oryza</i>
	AGACGCTGTGGAAGCGGCCGTT	<i>GA3ox1s</i> and <i>L.perrieriGA3ox2</i>
	CTAATACGACTCACTATAGGGCAAGCAGTGGTATCAACGCAG	For sequencing of <i>O.brachyanthaGA3ox1</i> and <i>O.granulataGA3ox1</i>
qRT-PCR target : <i>GA3ox1</i>	AGT	
	CTAATACGACTCACTATAGGGC	
	GTAAAACGACGGCCAG	For sequencing of pCR4 Blunt-TOPO vector
	CAGGAAACAGCTATGAC	
	TCAACATGTTCCCTAGGTGTCC	For <i>O.sativaGA3ox1</i> and <i>O.rufipogonGA3ox1</i>
	GAAGGTGAAGAAGCCTGAGTCC	
	ACGATTCATCTGAACATGTT	For <i>O.punctataGA3ox1</i>
	GAAGGTGAAGAAGCCCGAGT	
	GCTGCTCGCCATGTTCTT	
	ATCCGGACACCTGGGAAACAT	For <i>O.australiensisGA3ox1</i>
	GAGAGGCTGCTCTCCATGTT	
	TCCGGACACCTCGGAAACAG	For <i>O.brachyanthaGA3ox1</i>
	GACTACCACCGCTTCTGCTC	
	GTGAGCGCCTTGAAGAAC	For <i>O.granulataGA3ox1</i>
	GACTACCACCGCTTCTGCTC	
	CTTGGGGTACATGTTCAAGT	For <i>L.perrieriGA3ox1</i>
	GCCGGTGGTGGACATGAG	
	CCCACATTTGTTTCGGGTACC	For <i>O.sativaGA3ox1</i> / <i>osga3ox1</i> and <i>O.rufipogonGA3ox1</i> / <i>osga3ox1</i>
	GCCGGTGGTGGACATGAG	For <i>O.brachyanthaGA3ox1</i> / <i>osga3ox1</i>
	ACATGCGCTTCGGGTACC	
qRT-PCR target : <i>OsCIN3</i>	GCCGGTGGTGGACATGCG	For <i>O.granulataGA3ox1</i> / <i>osga3ox1</i>
	ACATGCGCTTCGGGTAGC	For <i>L.perrieriGA3ox1</i> / <i>osga3ox1</i>
qRT-PCR target : internal control (<i>UBQ5</i> gene)	CACATCCGCTTCGGGTACC	
	GCTTCAAGTCCGTGCACTCG	For <i>OsCIN3</i>
	CCGAGCTTCTTGCACAGC	
	AGCACAAGCACAAAGAGGTGAAG	For <i>UBQ5s</i> of <i>O.sativa</i> , <i>O.rufipogon</i> and <i>O.punctata</i>
	AGTAGTGGCGGTCCAAGTGGTT	
	CGCCGACTACAACATCCAG	For <i>O.australiensisUBQ5</i>
	CTTCTTCTTGCCTTCTTGG	
	CTACACCAAGCCCAAGAAGC	
	GCGTCGTGACCTTGTAGAAC	For <i>O.brachyanthaUBQ5</i>
	CTACACCAAGCCGAAGAAGC	
Expression and purification of <i>OsGA3ox2</i> and <i>OsGA3ox1</i>	GCGTCGTCAACCTTGTAGAAC	For <i>O.granulataUBQ5</i>
	ACCCTCGCCGACTACAACAT	
	CTCCTTCCTGAGGCGAGTAA	For <i>L.perrieriUBQ5</i>
	CCGGATCCATGACATCGTCG	
	CCCCCGGGCTAACTCTCCTTG	For <i>GST-OsGA3ox1</i>
	CCGGATCCATGCCGACGC	
	CCGAATTCTTATGCGTGAGCG	For <i>GST-OsGA3ox2</i>
	AACATGTACCCTAGGTGTCCAGATCCA	
	CCTAGGGTACATGTTGAGGTGAATCGT	For mutagenize <i>GST-OsGA3ox1</i>

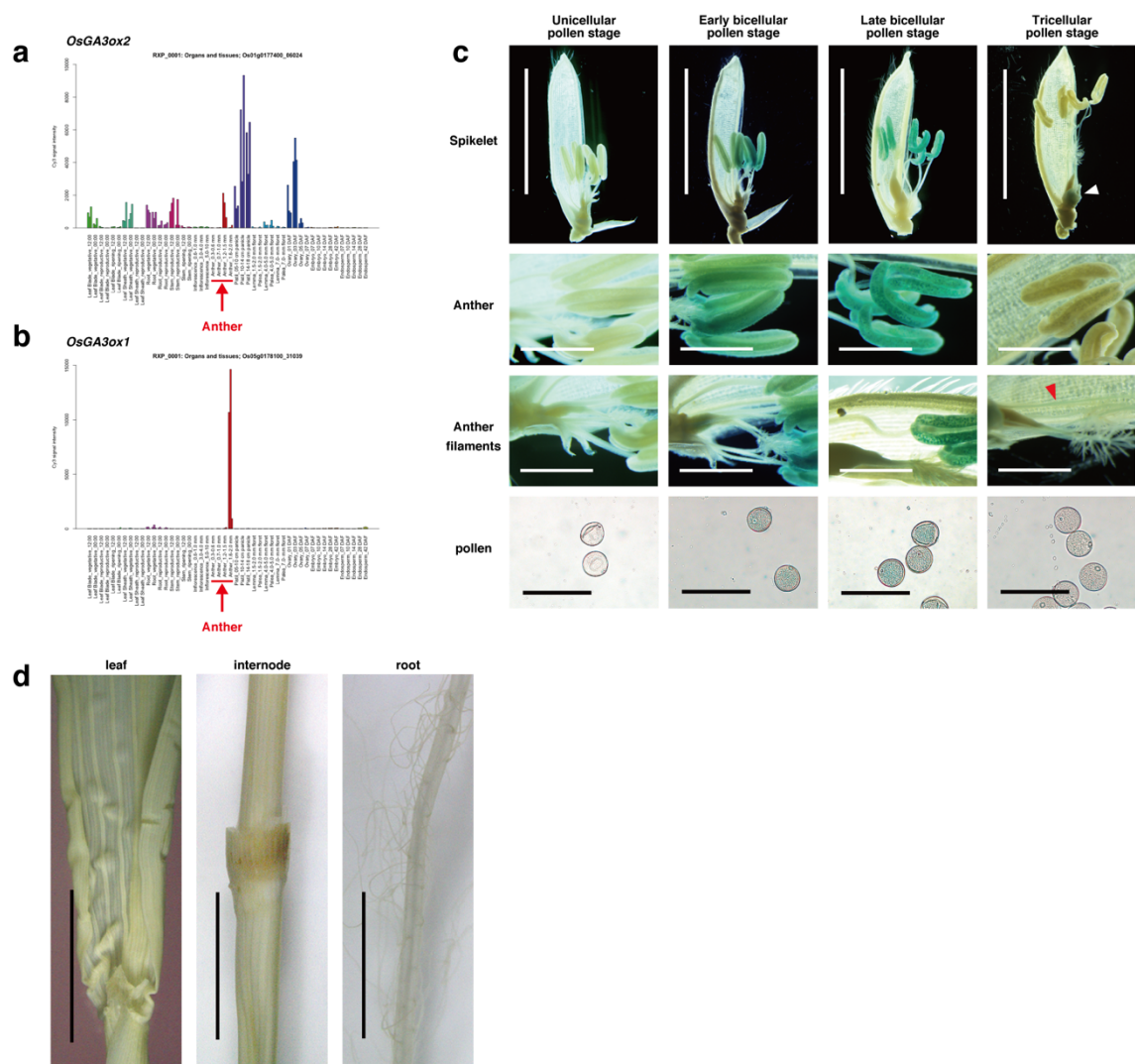
Supplementary Table 3. Primer and guide RNA list used in this study.

Supplementary Figures

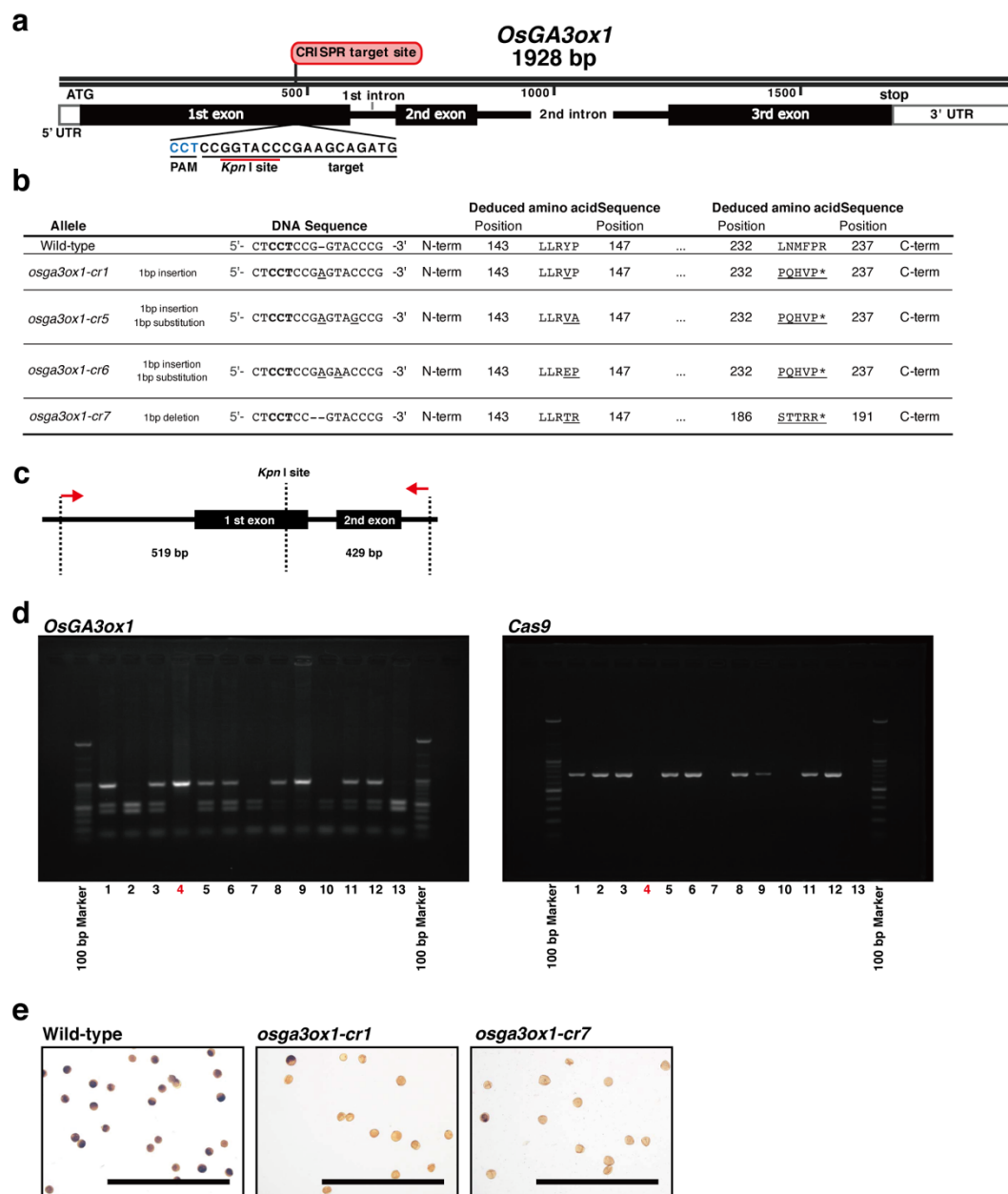


Supplementary Figure 1. Gibberellic acid synthesis pathway and its bioactivities. a) The GA synthesis pathway. Rice possesses the GA 13- and non-13-hydroxylation pathways, and GA13ox adds -OH to C13 of GA precursors. GAs are converted to the active form by GA3ox in the final step of GA

synthesis. C20-GA2oxs inactivate upstream GA precursors, and C19-GA2oxs inactivate bioactive GAs (GA₁ and GA₄) and the direct precursors (GA₉ and GA₂₀). **b)** The structure of bioactive GAs. GA₁ and GA₃ are 13-hydroxylated GAs that have undergone 13-hydroxylation (circled in green). GA₄ and GA₇ are non-13-hydroxylated, without 13-hydroxylation. Since GA₃ and GA₇ possess a double bond between the C1 and C2 atoms (circled in blue), they are not inactivated by GA2ox. **c)** Plant heights of GA₁, GA₃, and GA₇ treated Nipponbare and *slr1* mutant. For the *slr* mutant, homozygous individuals were selected at 13 DAGs, and only these were measured. ****: $p < 0.00001$, *****: $p < 0.000001$, n.s.: not significant. $n = 7$, (only in *slr* mutant, $n = 6$ (6 and 13 DAG) or 4 (20 and 27 DAG) because of withering). All p-values are listed in Supplementary Data 2 **d)** Plant of GA₁, GA₃, and GA₇ treated Nipponbare and *slr1* mutant. Red arrowheads in *slr* mutant plants indicate homozygous mutants. The *slr1* mutant had withered for about 13 days. bars = 30 cm. **e)** Induction on second sheath length of Tan-Ginbozu seedlings by bioactive GAs ($n = 13$ for each bioactive GA). Error bars = s.d. ****: $p < 0.00001$, *****: $p < 0.000001$, *****: $p < 0.0000001$, n.s.: not significant. **f)** Analysis of GID1 and SLR1 binding at various concentrations of GA by using yeast two-hybrid systems. Yeast strains were grown on -His plates for 7 days.



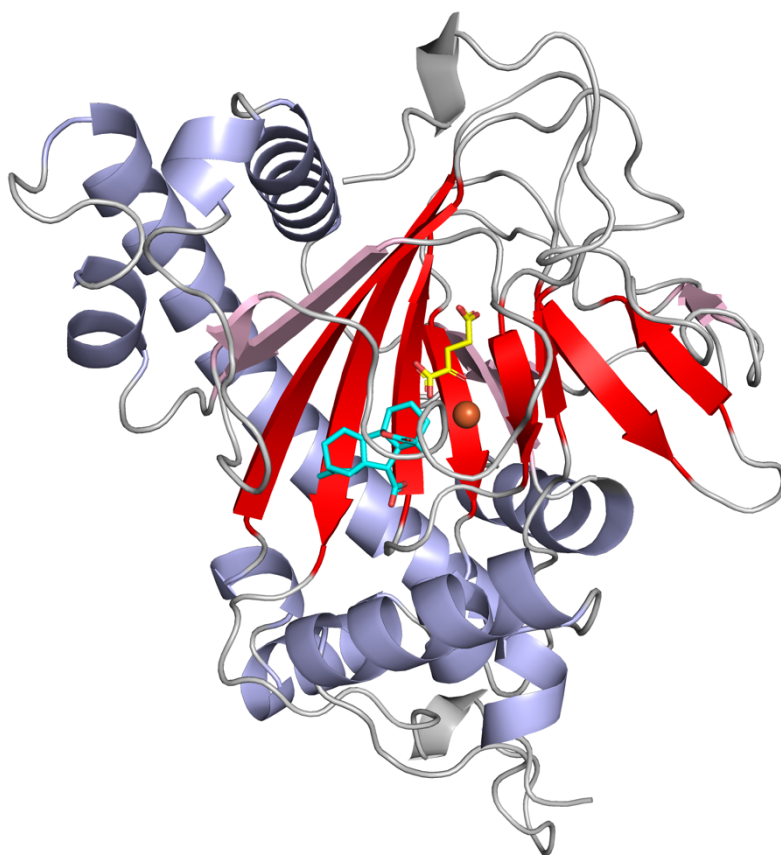
Supplementary Figure 2. Expression profiles of *OsGA3ox1* and *OsGA3ox2*. **a** and **b**) Tissue- and organ-specific expression profiles of *OsGA3ox2* (LOC_Os01g08220) (**a**) and *OsGA3ox1* (LOC_Os05g08540) (**b**) in rice based on data obtained from the RiceXpro database (<https://ricexpro.dna.affrc.go.jp>). **c**) GUS expression analysis. *OsGA3ox1* promoter-GUS was introduced into Nipponbare plants. Anther filament is indicated using a red arrowhead, and lodicules is depicted using a white arrowhead. Bars = 5 mm (spikelets), 1 mm (anthers), and 100 μ m (pollen grains). **d**) GUS activity in vegetative organs with the same transformants as that observed in **c**. No GUS activity was observed in vegetative growth organs. Bars = 5 cm (leaf, internode, and root).



Supplementary Figure 3. Generation of *osga3ox1* knockout mutants by using CRISPR/Cas9. a)

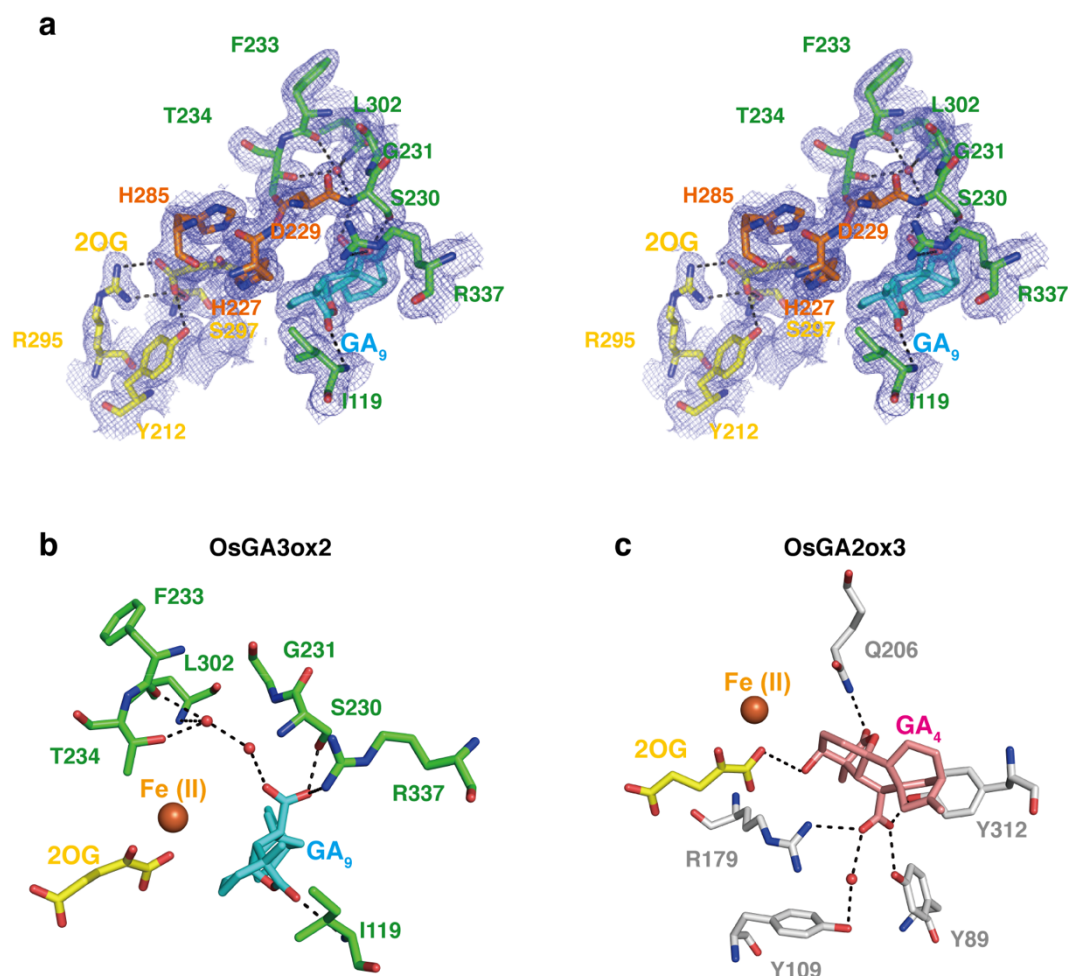
Diagram of the CRISPR/Cas9-mediated mutation introduced in *OsGA3ox1*. The white boxes, black boxes, and lines indicate the UTRs, exons, and introns, respectively. **b)** Modification of *OsGA3ox1* sequence conducted by using CRISPR/Cas9 in the T₀ generation. **c)** Schematic of genotyping of *osga3ox1* mutants analyzed by PCR and *KpnI* digestion. The size of the PCR product is 948 bp, which is digested into two fragments of 519 and 429 bp in Nipponbare (wild type) but remains intact in the case of the *osga3ox1* mutant. Gene sequences of the *osga3ox1* mutants were confirmed by sequencing. **d)** Genotyping of *osga3ox1* and its internal *Cas9* using gDNA from leaf blades of T₁ individuals; those with the *osga3ox1* mutation and *Cas9* missing from the genome are indicated in red letters. **e)**

Phenotypic analysis by KI staining of pollen from different alleles of *osga3ox1* mutant. Bars = 0.5 mm.



Supplementary Figure 4. Structure of OsGA3ox2 with substrates. Crystal structure of OsGA3ox2.

The α -helices are shown in blue, the common core fold is shown in red, and non core β -strands are shown in pink. 2OG and GA₉ are shown as sticks and colored by atom with carbons in yellow and cyan, respectively. Fe (II) is colored in orange.



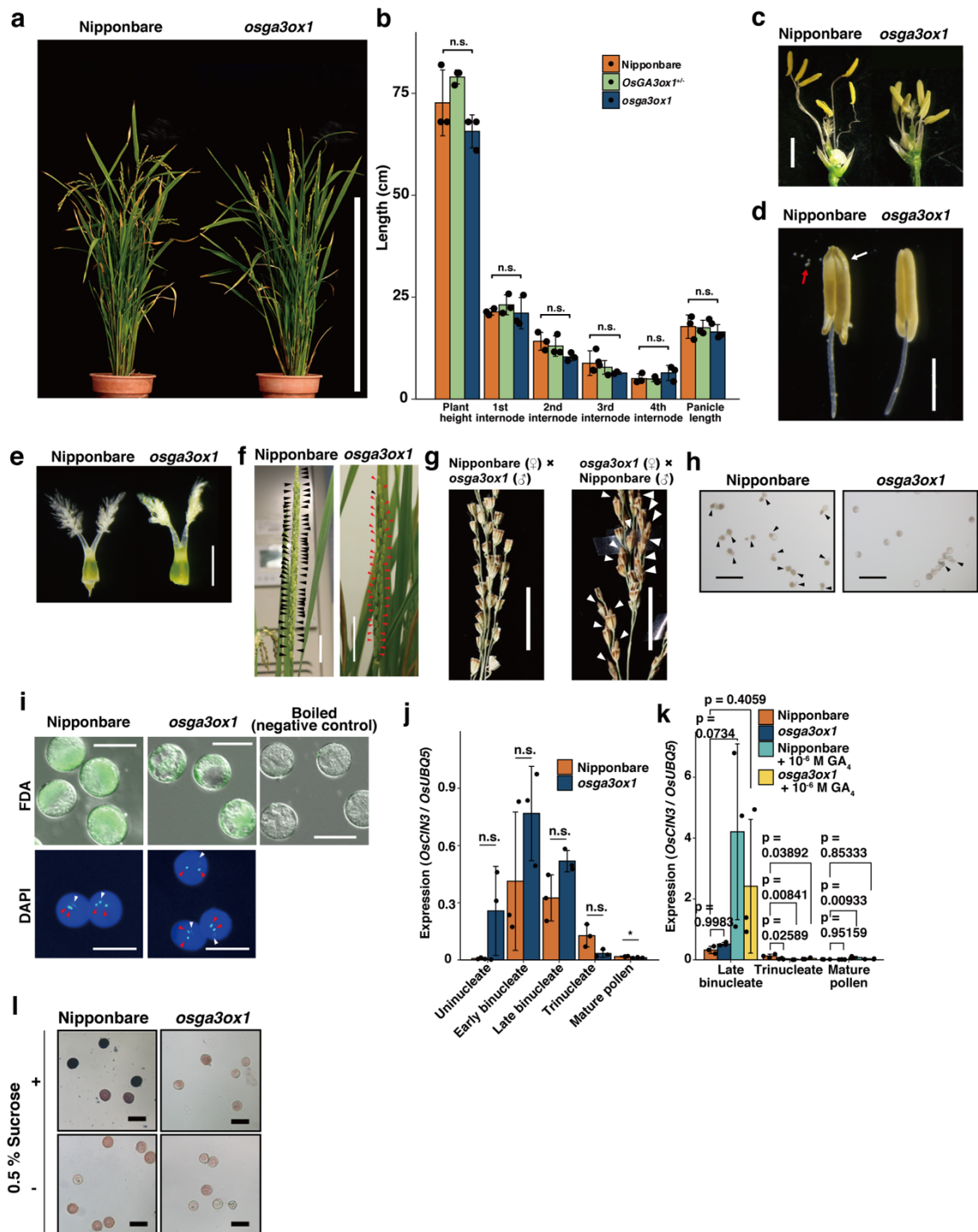
Supplementary Figure 5. Stereo views of the OsGA3ox2 active site and structural comparison of the active sites of OsGA3ox2 and OsGA2ox3. a) 2Fo-Fc electron density map contoured at 1.0 σ . The amino acids establishing interactions with Fe (II), 2OG, and GA₉ are indicated in orange, yellow, and green, respectively. The water molecules and hydrogen bonds are indicated by using red spheres and dashed lines, respectively. **b** and **c**) Close-up view of the structure of OsGA3ox2 (**b**) and OsGA2ox3 (**c**)⁸ around the GA-binding site with GA₉ (blue, OsGA3ox2) and GA₄ (pink, OsGA2ox3) and co-substrate, Fe (II) (orange), and 2OG (yellow). The amino acids establishing interactions with GA₉ in OsGA3ox2 (I119, S230, G231, F233, T234, L302, and R337) and GA₄ in OsGA2ox3 (Y89, Y109, R179, Q206, and Y312) are indicated in green and white, respectively.

OsGA3ox1	1	MOI-----	-----MTSSSTSPTSPLAAAAADNGVAAAYFN--	32
OsGA3ox2	1	MP-----	-----TPSHLKNPL-----CFD--	14
AtGA3ox1	1	M-----	-----PAMLTDFVRGHPHILP-----HSHIPD--	23
AtGA3ox2	1	M-----	-----SSTLSDVFRSHPIHIP-----LSNPPD--	23
AtGA3ox3	1	M-----	-----SSVTQLFKNNPVNRD-----RIIPLD--	22
AtGA3ox4	1	M-----	-----PSLAE-----EICIGN--	12
OsGA2ox1	1	M-----	-----VVPSATTPARQETVVA-----	17
OsGA2ox2	1	MGWDEPTFRGVWLRGEWDGLVPGEYSSQIRDQL-----	-----IGENV-----ADELVPPGTSERRDDAFLPRSR-----	61
OsGA2ox3	1	M-----	-----VVLAGPPAVDHIPLLRSP-----	19
OsGA2ox4	1	M-----	-----VVLAKPAALEQISLVRSR-----	19
OsGA2ox5	1	M-----	-----EEHDYD--	7
OsGA2ox6	1	M-----	-----PAFAD--	6
OsGA2ox7	1	M-----	-----VVLAK-GELEQIAL-----	14
OsGA2ox8	1	M-----	-----VAITAPSSIEHIPLVRCP-----	19
OsGA2ox9	1	M-----	-----PAIAD--	6
OsGA2ox10	1	M-----	-----VVLAK-GELEQIAL-----	14
OsGA2ox1	1	MSM-----	-----VVQOE-----QEVVFDAA--	16
OsGA2ox2	1	MVAEHP-----	-----TPPQPHQPPMDST-----AGSGI-----AAPA-----AAAVCD--	35
OsGA2ox3	1	M-----	-----AAVVFDA--	9
OsGA2ox4	1	MHA-----	-----SPHPLQIA-HDTLLSLTHTLCTAETGSTIRTGTAMVYISNAQDASKLIVTAKGGGGEADDAASS-----AAVULD--	74
OsGA3ox1	33	-FRGAERPESHVWKG-----HEKDT--APVAA-----	-----ADADGG-DAVPVVDSMGD-----DAAVAAVARAAEWE-----	90
OsGA3ox2	15	-FRAARRVPETHAWPGL-----DD--HEVVD-----	-----GGGGGGEDAVPVVDVGAGD-----AAARVARAAEQW-----	68
AtGA3ox1	24	-FTSLRELPSYKWT-----PKDDLFSAP-----	-----SPPATGENIPLIDLHPD-----ATNOIGHACRTW-----	78
AtGA3ox2	24	-F-----KSLPDSYTW-----PKDDLFSAS-----	-----ASDETLPLIDLSDIH-----VATLVGHACTW-----	71
AtGA3ox3	23	-FTNTKTLPSHVWS-----KEPE-----	-----TTSGPPIVILSNPE-----EHGLLRQACEW-----	68
AtGA3ox4	13	-LGSLOTLPESFTWKLT-----AADSLRPSSA-----	-----VSFDAVESIPVIDLSNP-----VTTLIGDASKTW-----	70
OsGA2ox1	18	-----AAPAAAA-----	-----SGVVGGGGVTTIATVDMSA-----ERGAVAROVATACAAH-----	60
OsGA2ox2	62	-----CSPSGATADSGDGSRLGDLGHDGDDDRG-----	-----GASAMARRIOWGGQRR-----	112
OsGA2ox3	20	-----DEGDV-----	-----FSGVPVVDLGSFG-----AARAVVDACERY-----	49
OsGA2ox4	20	-----SVED-----	-----NFGAGLPVVDLAADG-----AAGEVVRACERF-----	50
OsGA2ox5	8	-SNSNPPLMSTYKHLFVEQHRLDM-----	-----DMGAIDVDECELPVIDLAGLM-----EAEQVCRAADMVRAASEW-----	68
OsGA2ox6	7	-IAIDPPLADSYRALALLRRDRDGGIAPPAVQM-----	-----VGSGGAVLERDLPMVDLERLT-----RGGAGERKACAGAMARAASEW-----	80
OsGA2ox7	15	-----PAAH-----	-----PPPADVRADLSATG-----PARAAEARALVAACEEQ-----	50
OsGA2ox8	20	-----KGANA-----	-----GPQAVIPCIDLSAPG-----AAAADACRTL-----	51
OsGA2ox9	7	-CAADPLADSYTLLRLGGDDDD-----DACTKV-----	-----TTTPQPVSECELPMDVGCILTAPTGAATAAAVQGGHQAEEERACAAIAAAAEW-----	90
OsGA2ox10	15	-----PAAA-----	-----PPLADVPEVDLGGGA-----CRADAARAVVAACEGH-----	49
OsGA2ox1	17	VLSGQTEIPSQFIWP-----AEE--SPGSV-----	-----AVELEVALIDVGAGA-----ERSSVVRQVGEACERH-----	71
OsGA2ox2	36	-LRMEPKIPEPFVWP-----NGDA--RPASA-----	-----AELDMPVVDVGVLV-----DGDAEGLRRAAOVAAACATH-----	93
OsGA2ox3	10	ILSQEALPAQFVWP-----ADE--APAAD-----	-----DGVVEEIAIPVVDLAAPL-----ASGGIGRDVAEACERH-----	66
OsGA2ox4	75	LWRQPAKIPAFVWP-----RADV--ALPSS-----	-----PTGELDVPVVDLAAAL-----R--DAAGMRRVAQVAAACASH-----	136
OsGA3ox1	91	-----CGLLVGHGVTA-----	-----ALARVEAQAARLEALPADDKARGARR-----PG--G-----GNTGYGVPPYLLRYPKQMWAEYGTFFPP--	160
OsGA3ox2	69	-----CAFLVGHGVPA-----	-----LLSRVEERVARVSLPASEKMRVRG-----PG-----EPCGYGSPPLSSFFSKLMWSEGYTFSPS--	137
AtGA3ox1	79	-----CAFQISNHGVPLG-----	-----LLQDIEFLTGSLFGLPVQRKLKSARS-----ET-----GVSGYGVARLASFFNKMWSEGYTFVGS--	147
AtGA3ox2	72	-----CAFQITNHGVPSR-----	-----LLDDIEFLTGSLFGLPVQRKLKAARS-----EN-----GVSGYGVARLASFFNKMWSEGYTFVGS--	140
AtGA3ox3	69	-----GVFHITDHGVSHS-----	-----LLHNVDQCMKRLFSLPMHRKILARSS-----PD-----ESTGYGVRRSMFYDKLMWSEGYFVMSG--	137
AtGA3ox4	71	-----CAFQIANHGISQK-----	-----LLDDIESLSKTLFDMPSERKLEAASS-----DK-----GVSGYGEPRSPFFEKMWSEGYTFIAD--	139
OsGA2ox1	61	-----GFFRCVGHGVPAAP-----	-----VAARLDAATAAFFAMAPAEKORAG-----PA-----SPLGYGCRSLG--NGDVGELEYLLHAN--	128
OsGA2ox2	113	LIRRWGGLWRWIRRRGGAAACEEVEGRRIRRWGLRLWLRSSRSGSGGVRGGRGGAADPAMGGAATAPFLALAHRLFL-----	-----LLLLAAG--	203
OsGA2ox3	50	-----GFFKVNHGVATD-----	-----TMDKAESAVRFESOTOPDKDRSG-----PA-----PFGYGSKRGLG--NGDMGWLEYLLALD--	115
OsGA2ox4	51	-----GFFKVVSHGVGEG-----	-----VVGRLAEAVRFPASPOAKDAHG-----PA-----SPFGYGSKRGLG--NGDMGWLEYLLALD--	116
OsGA2ox5	69	-----GFFQVTHNGVPOA-----	-----LLRELHDAQAVARRRFPQEKVTERLL-----GF-----SPESYRWGTPTAKC--LEQLSWSEAYHIPMT--	138
OsGA2ox6	81	-----GFFQLTNHGVGRE-----	-----LMEEMRRQARLRLPFPETKEKAGLL-----N-----GSYRWGNPTATS--LRHLSWSEAFHVPLA--	147
OsGA2ox7	51	-----GFFRVTHGVPPG-----	-----LVRAAEAAAARFFALPQPDKEAAA-----G-----APLGYASKRGLS--AGDLGWLEYLLALD--	115
OsGA2ox8	52	-----GFFKATNHGVAPG-----	-----LADALESSAMAFALPQHEKLDMSG-----PA-----RPLGYGSKRGLS--NGDVGWLEYLLASG--	118
OsGA2ox9	91	-----GFFQVNVHGVPAE-----	-----LLEAMRREQARLRLPFPFAKSSAGLL-----N-----DSYRWGTPTATS--LRQLSWSEAFHPLA--	157
OsGA2ox10	50	-----GFFKVTCHGVPAE-----	-----LLARVEAAAAAFPAQMEKEAASS-----AAAAPG-----SPFGYGSKRGLS--NGDLGWLEYLLGVA--	122
OsGA2ox1	72	-----GFFLVNVHGEIA-----	-----LLEEAHRCMDAFTLPLGEXQARQR-----AG-----ESCGYASSFTGRFASKLPWKETLSFRYS--	140
OsGA2ox2	94	-----GFFQVSEHGVDA-----	-----LARAALDGASDFRLPLAEKRRARRV-----PG-----TVSGYTSAHADRFASKLPWKETLSFGPH--	162
OsGA2ox3	67	-----GFFQVNVHGVDP-----	-----LLAEAYRCDDAFYARPLAEKQARRR-----PG-----ENHGYASSFTGRFCKLPWKETMSFNCS--	135
OsGA2ox4	137	-----GFFQVSHGVGPS-----	-----LARAALDGAAGFRLPFAAKQARRA-----PG-----TVTGYTAAHADRFVNDLPWKETLSFGHR--	205
OsGA3ox1	161	AIRDE-----	-----FRRVWPDAGDDY-----HRFCSAMEEYDSSMRALGERLLAMFFKAGLAGND-----APGGET-----ERKIRETLTSTIHLMYPRCP--	239
OsGA3ox2	138	SLRSE-----	-----LRLRLPKSGDDY-----LLFCVMEEFHKEMRLADELLRLFLRALGLTGEE-----VAGVEA-----ERRIGERMATVHLMYPRCP--	216
AtGA3ox1	148	PLND-----	-----FRKLWP-----QHH-----LNYCDIVEEYEEHMKLASKLMWLALNSGVSEED-----IEWAS-----LSSDLNWAQAALQNLHMYVCP--	221
AtGA3ox2	141	PLHD-----	-----FRKLWP-----SHH-----LKYCEIIEEYEEHMKLAKLMWALGSGVGEKD-----IQWAG-----PNSDFQGTQAVIOLHMYKCP--	214
AtGA3ox3	138	SLRRH-----	-----ATLLWP-----DDH-----AEFCNVMEEYOKAMDDLSHRLISMLMGLTHED-----LGWLVPDKTGSQDTSIQSFLOLHMYVCP--	215
AtGA3ox4	140	SYRHH-----	-----FNTLWP-----HDH-----TKYCGIIQEVVDEMEKLSRLLYCILGSECVTVED-----IEWAHK--LEKSGSVGRGAIRLHMYVCP--	216
OsGA2ox1	129	PAVAH-----	-----RARTIDAMD-----SRFSAIVNEYIEAMKKLACEILDLLGEGGLKDP-----YFSKL-----TTN-----ADSDCLLRINHMYPSC--	203
OsGA2ox2	204	SSSCSSSGDGEDCGRSGDRLTESLFVDIGLESDCRQVNDYEAVRQLACHVLDLLGEGGLRDPT-----	-----SLTRL-----ITA-----TDNDSLIRINHMYPSC--	294
OsGA2ox3	116	DASLA-----	-----DACTVPSC-----AVFRAALNEYISGVRKVAVRVMEAMSEGGAQAD-----ALSAL-----VTA-----EGSDQVFLRVNHMYPCR--	187
OsGA2ox4	117	GASLS-----	-----SSRLDAANKYVGAMRGMAVTVLEMVABEGGVAPRG-----ALADM-----VVGDAASDQILRLHMYPCR--	189
OsGA2ox5	139	TPRPSTS-----	-----RSSPAPS-----IRARAVIEEVSAMYELAQKLAEILMRGPGAGEG-----ETMVT-----TREETCFLLRLHMYPCA--	202
OsGA2ox6	148	SISGADCF-----	-----GDL-----TSLRGVMQEVAEAMSRVANTVAAALAEETGRRGG-----GASAAP--WFPAGCDETTCFLLRLHMYEACP--	222
OsGA2ox7	116	PAAAAA-----	-----LPCAATSPTPP-----CPLRELLREYSAVRRVACGVLELMAEGGVGPAD-----ALARL-----VAR-----EDSDSLRVNHMYPRP--	192
OsGA2ox8	119	AASSG-----	-----GAALP-----AALRAAVEAYTGAVRGVGRVMEAMAEGLGASEEGRCVLRM-----VVG--CEGSDMLRVNHMYEPL--	192
OsGA2ox9	158	GISGKSCNY-----	-----GDL-----TSLRDVTRREVADMSRLARALARVLAESLGHAAAG-----ER-----FPEGCDATDQLRLHMYPCR--	227
OsGA2ox10	123	AAAAAPL-----	-----PAHGEASPSY-----GSFRDILNEYVAVRAMMEVLKMAEGGLKEKD-----ALVRL-----VSH-----EESDSVLRVNHMYPPH--	200
OsGA2ox1	141	SAGDEEG-----	-----EEGVGEYLVRKLGAEHG-----RRLGEVYSRYCHEMSRLSELMEVLGESGIVGDR-----RHY-----FRRFFQRNDSIMRLHMYEACQ--	223
OsGA2ox2	163	DRAAA-----	-----PVVADYFSTLGPDP-----APMGRVYQKYEEMKELSLTMELELSGVERGY-----YREFFADSSSIMRCNYEPCP--	238
OsGA2ox3	136	AAPGN-----	-----ARMVADYFDALGEY-----RHMGEVYQYECVDMVTRIALDVTVELVAVGLGRGE-----LRGFFADGDVPMRLHMYEPCP--	212
OsGA2ox4	206	HANAAGN-----	-----NSSSTVADYF--STLGDDF-----KHLGEVYQYECAMQEEVTKAIMAVLGESGIVGGY-----YREFFEDSSSIMRCNYEPCP--	284

OsGA3ox1	240	DEP-----RVVGLAAHTDSGFFTFI--LQS-PVPGLOLLRH-----RPDRWVTVF--GTFEGALIVVGDLFHVLITNG	301
OsGA3ox2	217	EPR-----RALGLIAHTDSGFFTFV--LQS-LVPGLOLFRR-----GPDWVAVF--AVAGAFVNVGDLFHILITNG	278
AtGA3ox1	222	EPD-----RAMGLAAHTDSTLLTIL--YQN-NTAGLOVFR-----DDLGMVTVF--PFGSLVNVGDLPFILHSNG	282
AtGA3ox2	215	EPD-----RAMGLAAHTDSTLLTIL--YQN-NTAGLOVFR-----DDVGMVTVF--PFGSLVNVGDLLHILITNG	275
AtGA3ox3	216	DPH-----LAMGLAPHTDSLLTIL--YQG-NIPGLEIESPQE--EGSRMIGVE--PIEGSLVNVGDLSHIIISNG	279
AtGA3ox4	217	EPE-----RAMGLAAHTDSTLLTIL--HQS-NTGGLVFR-----EESGWVTVF--PAPGVLVNVGDLPFILHSNG	277
OsGA2ox1	204	NIHKL-DHDDQCNIKSLVSTKASNGNLMAGGRICFGEHSDPQILSL--RAN-DVEGLVFPVDP--HEGKEMVVOVP--SDPSAIFVNVGDVLQALITNG	295
OsGA2ox2	295	AAAAG-DHK-----SGGGPAPTAAICFGEHTDPQILSVL--RAN-DADGLQLLLPDAAAAGDSVWVPVP--PDPSAFFVNVGDLQALITNG	374
OsGA2ox3	188	ALQGL-G-----SVTGFGEHTDPQILSVL--RSN-GTSGLOIALR-----DGQWVSVF--SDRDSFFVNVGDSLQVLITNG	252
OsGA2ox4	190	PLLQN-LMP-NC-----SPTGFGEHTDPQILSIL--HSN-STSGLOVALHHDADAGDHQWTVF--PDPAFLVNVGDSLQVMTNG	263
OsGA2ox5	203	MAM-----GGFCLCPHTDSDLTIVHQQD-TVGGLOLL-----KGGRWVAVK--PSESTLIVNVGDLQAWNSND	264
OsGA2ox6	223	FAA-----DTFGLVPHTDSDLTIVL--CQD-QVGGHLM-----KDSRWVAVR--PRDALVNVGDLQAWNSN	282
OsGA2ox7	193	DQLGGGGGP-----NLTFGEHTDPQIISVL--RSN-GAPGLEISLR-----DGAWASVPHDGDGDSFFVNVGDTLQVLITNG	261
OsGA2ox8	193	LFPGR-DRD-EC-----GVTFGEHTDPQIISVL--RSN-CTAGLOILLRGD-YSSPARWVPVP--PDPSFFVNVGDSLQVLITNG	265
OsGA2ox9	228	FPPD-----DAFGLVPHTDSDLTIVL--CQD-HVGGLOLM-----KGSRWVAVK--PIPGALVNVGDLQAWNSN	288
OsGA2ox10	201	ELKQ-Q-GHG-----RLTFGEHTDPQIISVL--RSN-DTSGLEISLR-----DGSWASVF--PDRKSFVNVGDLQVLITNG	266
OsGA2ox11	224	RPL-----DTLCTGPHCDPTSLTIL--HQD-HVGGLEIVW--AEGRWRAIR--PRFGALVNVGDTFMALISNA	283
OsGA2ox12	239	EPE-----RTLCTGPHCDPTALTIL--LQD-DVGGLEVL-----VDGWRPVS--PVEGAMVNICDTFMALISNG	298
OsGA2ox13	213	QPH-----LTLCTGPHCDPTSLTIL--HQD-DVGGLOVLPDDA-AAAAGRWRAVR--PRADAFVNVIGDTFAALITNG	278
OsGA2ox14	285	EPE-----RTLCTGPHCDPSALTIVL--LQGDVDGLQVL-----VAGWRPVR--PLEGAFVNVIGDTFMALITNG	345
OsGA3ox1	302	RFHSVYHRAVV-NRDRDRVSLGYFL--GPPADMKVTPPLVAAGS-PE-----SKAVYQAVTWPEYMAVRDKLFGTNISALSMIR-----VAKEEDKES--	384
OsGA3ox2	279	RFHSVYHRAVV-NRDRDRVSLGYFL--GPPDAEAVLPEAVP-AG-----RSPAYRAVTVPEYMAVRKKAFATGGSALKMVSTDAAAAADHDDVAAA	368
AtGA3ox1	283	LFKSVLHRAVV-NQTRARLSVAPLW--GQSDIKISPPVKLV-SV-----ESPLYQSVTWKEYLRTKATHFNKALSMIRNHR-----EE-----	358
AtGA3ox2	276	IFPSVLHRAVV-NHVRSRFSMAVLW--GPPSDIMISPLPKLV-DL-----QSPLYPSLTWKQYLATKATHFNQSLSIIRN-----	347
AtGA3ox3	280	QFRSTVHRAVV-NKTHHRVSAAYFA--GPPKNLQIGPLTS--D-KN-----HPPIYRRLIWEYLAAKATHFNKALTLFR-----	349
AtGA3ox4	278	KIPSVHRAVV-NHTRSRISIAYLWGGAGDVQIAPISKLTG-PA-----EPLSYRSITWKEYLQIKYEVDKAMDARVNVN-----	352
OsGA2ox1	296	RLISIRHRAVV-TACRPRLSTIYFA--SPLHARISALPETIT-AS-----SPRRYRSFTWAEYKRTMYSL--RLSHSRLELFK-----IDDDSDNASE	379
OsGA2ox2	375	RLVSIRHRAVV-GTGKPRLSIYFA--APPLHARISALPETVA-AG-----APRRYRAFTWAEYKRTMYTL--RLSHNRDLDFH-----AGDGDGDAGVG	458
OsGA2ox3	253	RFRSVKHRVVA-NSLKSRSVFIYFG--GEPQAQRIAPLQLLG-EG-----EQSLYKEFTWDEYKAAKKS--RLGDNRLAQFE-----KK-----	327
OsGA2ox4	264	RMRSVHRVVA-NKLKSRSVSMIYFG--GEPLEQRIAPLQLLV--AGVNGEESQSRYEFTWGEYKKAAYLS--RLSDNRLAPFH-----RQPPPVANPLA	354
OsGA2ox5	265	VYKSVHRVMA-NATLERFSMAFFL--CSYHTLIIPSSSHV--HD-----DDAHYRSFTWGEYKQIMEDVSTGRKIGLHR-----FRTR-----	341
OsGA2ox6	283	RYKSVHKVVA-NAKTDRLSVAYFL--CSYDSLVTG-----CG-----EPSPYRAFTWGEYRKKVQEDVRTTGKKIGLFPN-----FFKHSSVQ---	358
OsGA2ox7	262	RFRSVKHRVVA-NSEKRSVSMVFFG--GPPGERLAPLALLG-DG-----GRSRYREFTWKEYKSGCKG--RLADDRLCRFE-----N-----	335
OsGA2ox8	266	RFRSVKHRVLAPEGEESRLSVIYFG--GPAASQRIAPLEQVMR-EG-----EQSLYREFTWGEYKKAAYKT--RLGDNRLGPYE-----LQHAAANDEAA	350
OsGA2ox9	289	RYKSVHRVMT-NATTERSVAYFL--CSYDSPIGT-----CR-----EPSPYKAPTGEYRRRVQEDVKKTGKTGLSN-----FLV-----	359
OsGA2ox10	267	RFRSVHRVMT-SSPRPRSVIFFA--GPPPRERLAPLWVAEDG-----GRRRYREFTWREYKASAYRT--KLAENRLCHF-----TEAD-----	344
OsGA2ox11	284	RYRSCVHRAVV-NSTAPRRSLAFFL--CEMDTVVRPPEELVD-DH-----HPRVYPDFTWALLDFTQRHYRADMTLOAFS-----DWNLHHRHLOP	368
OsGA2ox12	299	RHASCVHRAVV-NQRERRSLAFFL--CRREDRVVRPPPSAA-----TPQHYPDFTWADLMRFTQRHYRADMTLODAFT-----RWLAPPAADAA	380
OsGA2ox13	279	RHASCVHRAVV-NGRVARRSLTFFL--NERLDRVVSPPPALVD-AA-----HPRAFPDTWRELFLEFTRHYRSDTNTMDAFV-----AWIKQRNGYES	363
OsGA2ox14	346	RYKSCVHRAVV-HREQERRSLAFFL--CRREDRVVRPPAGAG--AG-----ERRLYPDFTWADFMRTQRHYRADTRTLDAFA-----RWLRPPACSGA	429
OsGA3ox1	384	-----	384
OsGA3ox2	369	ADVHA-----	373
AtGA3ox1	358	-----	358
AtGA3ox2	347	-----	347
AtGA3ox3	349	-----	349
AtGA3ox4	353	-----PTN	355
OsGA2ox1	380	GKA-----	382
OsGA2ox2	459	DDDDHE-----	464
OsGA2ox3	327	-----	327
OsGA2ox4	354	-----	354
OsGA2ox5	341	-----	341
OsGA2ox6	358	-----	358
OsGA2ox7	335	-----	335
OsGA2ox8	351	TKK-----	353
OsGA2ox9	359	-----	359
OsGA2ox10	344	-----	344
OsGA2ox11	369	TIYS-----	372
OsGA2ox12	381	A-----TAQVEAAS-	389
OsGA2ox13	364	LDKY-----	367
OsGA2ox14	430	APVVGEPPTATQAATV	445

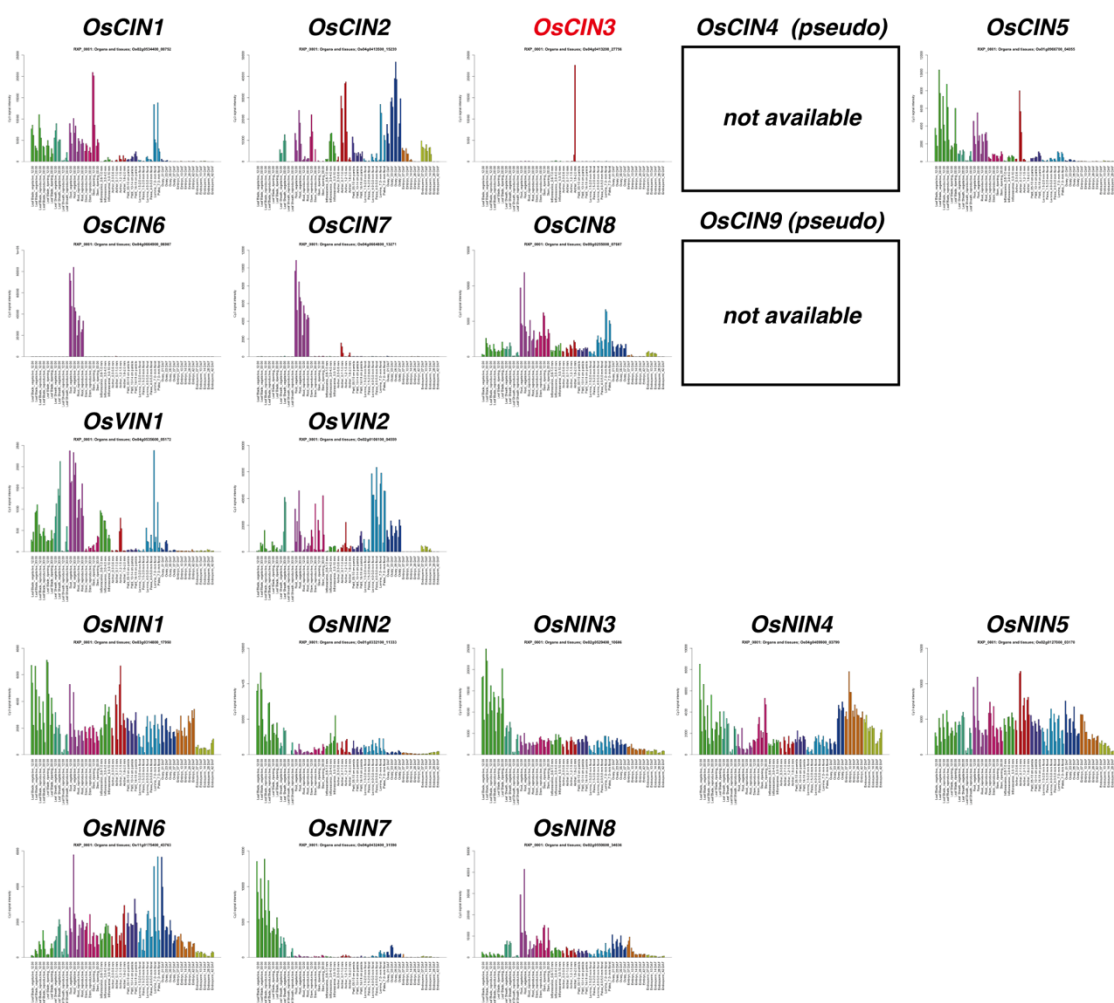
Supplementary Figure 6. Amino acid sequence alignment of OsGA3oxs AtGA3oxs, OsGA2oxs, and OsGA20oxs. Protein sequence data of OsGA3oxs, AtGA3oxs, OsGA2oxs, and OsGA20oxs collected from public databases (Supplementary Table 2) were aligned using MAFFT version 7.3 with the L-INS-i model⁹. Alignments are displayed using the GENETYX-MAC software (<https://www.genetyx.co.jp>). Residues with 100% identity are shown in black, and those with more than 50% identity are depicted in grey. The residues establishing interactions with Fe (II) and 2OG are highlighted in orange and yellow, respectively. Green-highlighted residues indicate the conserved residues that were determined by considering the crystal structure of OsGA3ox2 for binding to substrate GA₉. Regarding OsGA2ox3, the residues highlighted in blue and red represent the residues binding to GA₄ in the active site and intermolecular binding site, respectively, as shown previously by

Takehara et al.⁸. Species abbreviations: Os, *Oryza sativa*; At, *Arabidopsis thaliana*.

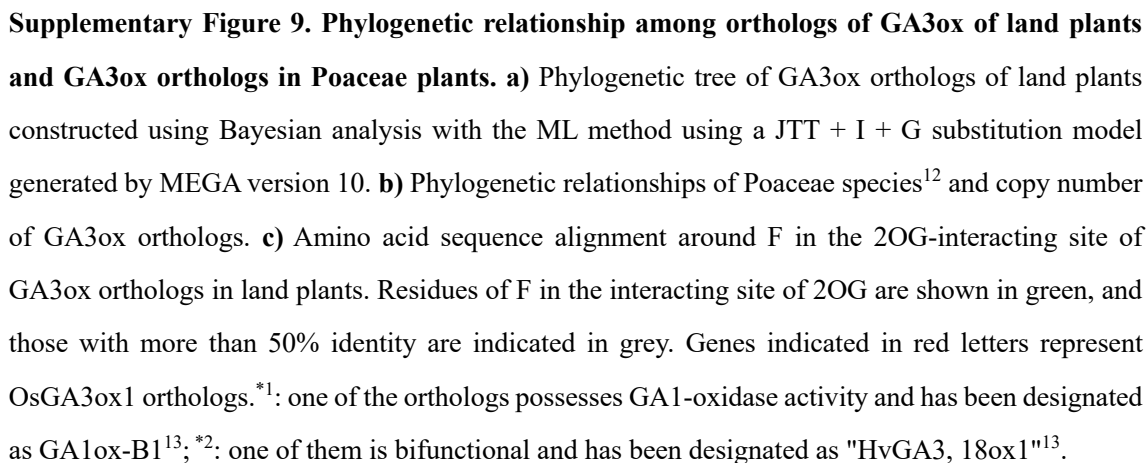


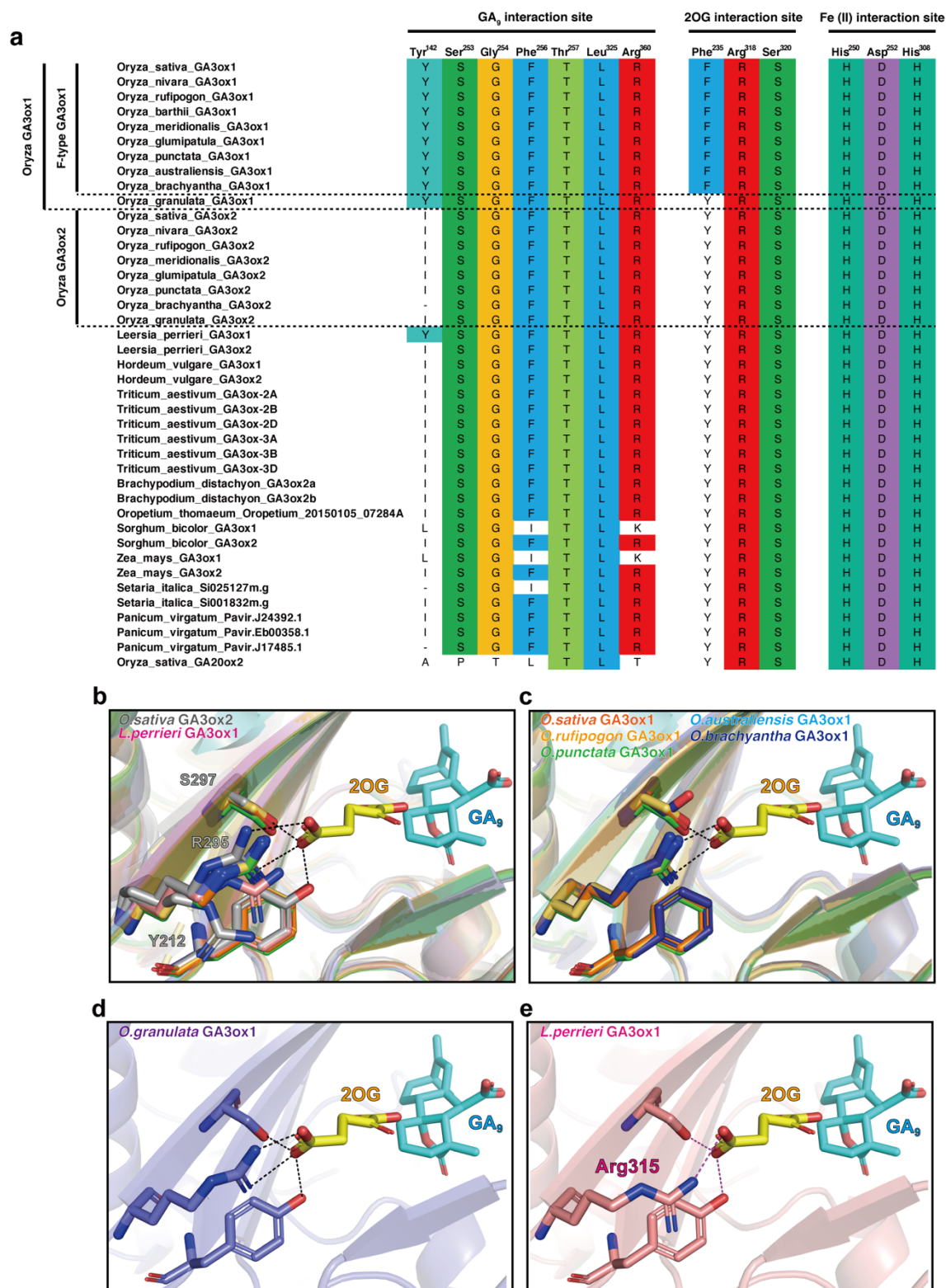
Supplementary Figure 7. *osga3ox1* knockout rice are physiologically abnormal in the flowering stage. **a)** Vegetative phenotype of Nipponbare (left) and *osga3ox1* knockout mutant (right). Bar = 50 cm. **b)** Evaluation of aboveground phenotype of Nipponbare, *OsGA3ox1*^{+/-} (heterozygous), and *osga3ox1* mutant (homozygous). Error bars, s.d. n = 3, one-way ANOVA. n.s.; not significant (p > 0.05). **c-e)** Phenotype of the flowering spikelets with palea and lemma removed (**c**), anthers (**d**), and pistils (**e**) of Nipponbare and *osga3ox1* mutant. Red and white arrows indicate pollen grains and anther

dehiscence, respectively in **d**. Bars = 2 mm (**c**), 1 mm (**d** and **e**). **f**) Observation of flowering of Nipponbare and *osga3ox1* mutant panicles. Black and red arrowheads indicate opened and non-opened spikelets, respectively. Bars = 2 cm. **g**) Seed fertility of pistils upon reciprocal crossing test of Nipponbare pistils with *osga3ox1* mutant pollen (left) and *osga3ox1* mutant pistils with Nipponbare pollen (right) as described in Figure 2f. White arrowheads indicate seed setting. Bars = 2 cm. **h**) *In vitro* pollen tube germination of Nipponbare and *osga3ox1* mutant. Black arrowheads indicate pollen tube germination. bars = 100 μ m. **i**) Staining of pollen grains in Nipponbare and *osga3ox1* mutant using FDA and DAPI for cell viability. Boiled pollen grains were used as a negative control (“Boiled”). Both wild-type and *osga3ox1* mutant pollen grains showed green fluorescence, indicating that the cells were alive. Red and white arrowheads indicate sperm nuclei and vegetative nucleus, respectively. Bars = 50 μ m. **j** and **k**) qRT-PCR expression analysis of *cell-wall invertase 3 (OsCIN3)* in anthers of Nipponbare and *osga3ox1* mutant (**j**) or those treated with GA₄ (**j**). Error bars, s.d. n = 3, two-tailed paired *t*-tests (****p* < 0.001, ***p* < 0.01, **p* < 0.05) (**i**), and *p*-values by Dunnett’s multiple comparison test (**k**) are indicated. **l**) *In situ* stains of acid invertase activity of the Nipponbare and *osga3ox1* mutant pollen grains using nitroblue tetrazolium. Upper panels: + sucrose; lower panels: control (– sucrose). Pollen grains stained black possess invertase activity. Bars = 50 μ m.



Supplementary Figure 8. Expression profiles of rice invertases. The tissue- and organ-specific expression profiles of nine *cell wall invertases* (CINs), eight *neutral invertases* (NINs), and two *vacuolar invertases* (VINs) obtained from the RiceXpro database (<https://ricexpro.dna.affrc.go.jp>). *OsCIN4* and *OsCIN9* are pseudogenes^{10,11}.



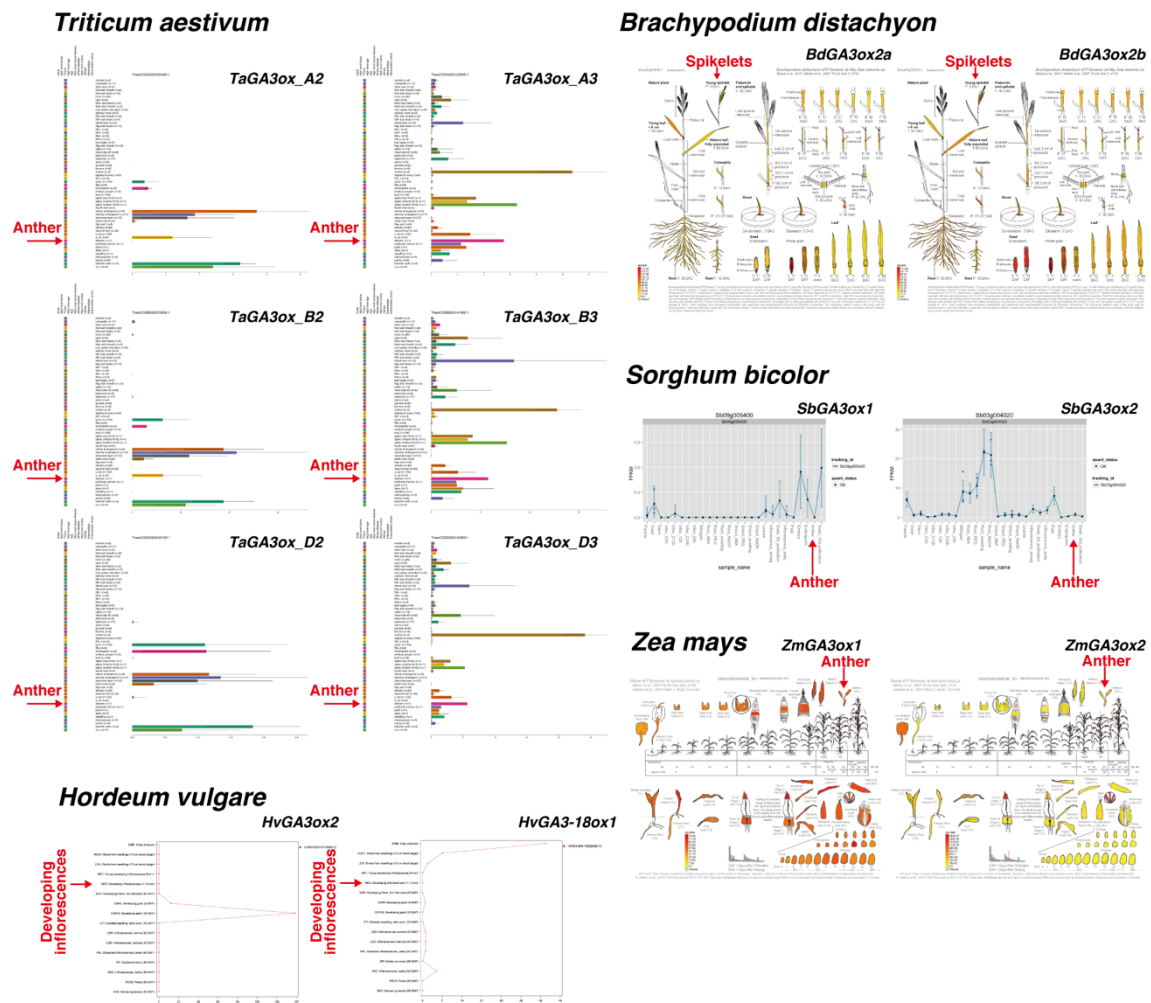


Supplementary Figure 10. Substrate interaction site of *Oryza* and *L. perrieri* GA3ox1. a) Alignment of GA₉, 2OG, and Fe(II) interacting site of Poaceae GA3ox orthologs. The residue numbers are those of OsGA3ox1 determined by the alignment analysis (Supplementary Figure 6). For *Oryza*

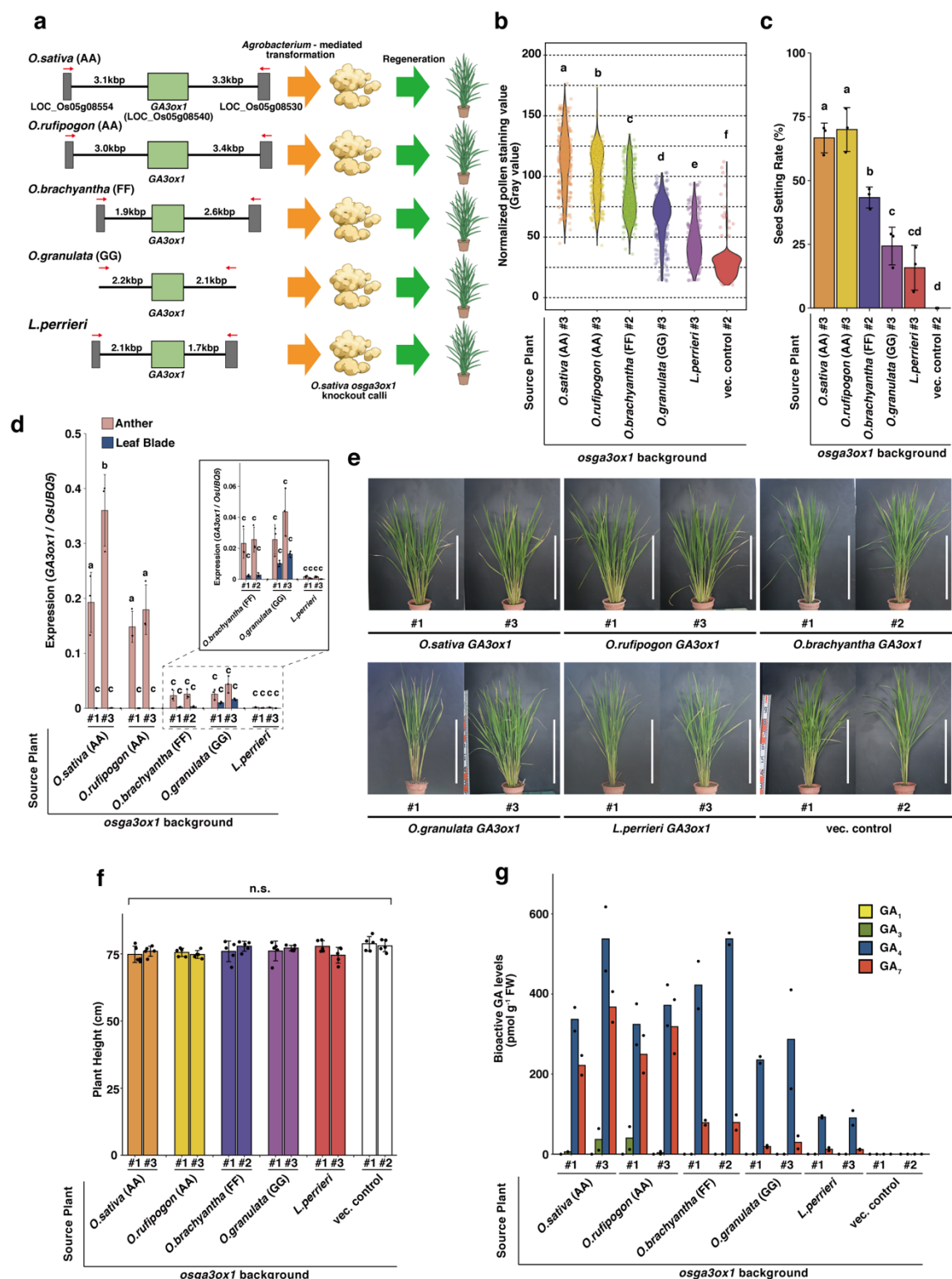
GA3ox1 orthologs, (co-) substrate interaction residues except Phe235 are conserved. **b-e)** Close-up view of the active site structure around 2OG in *Oryza* and *L. perrieri* GA3oxs as predicted by AlphaFold2 algorithms¹⁴

(<https://colab.research.google.com/github/sokrypton/ColabFold/blob/main/AlphaFold2.ipynb>).

Superimposed structures of *Oryza* and *L. perrieri* GA3ox1 and OsGA3ox2 (**b**) and *O. sativa*, *O. rufipogon* (AA), *O. punctata* (BB), *O. australiensis* (EE), *O. brachyantha* (FF) (**c**). The hydrogen bonds interactions of 2OG with residues of OsGA3ox2 (**b**) and *O. sativa* (**c**) are displayed. **d** and **e**) The structure of *O. granulata* (GG) (**d**) and *L. perrieri* (**e**). 2OG and GA₉, are shown with yellow and cyan sticks, respectively. Hydrogen bonds are indicated by dashed lines.

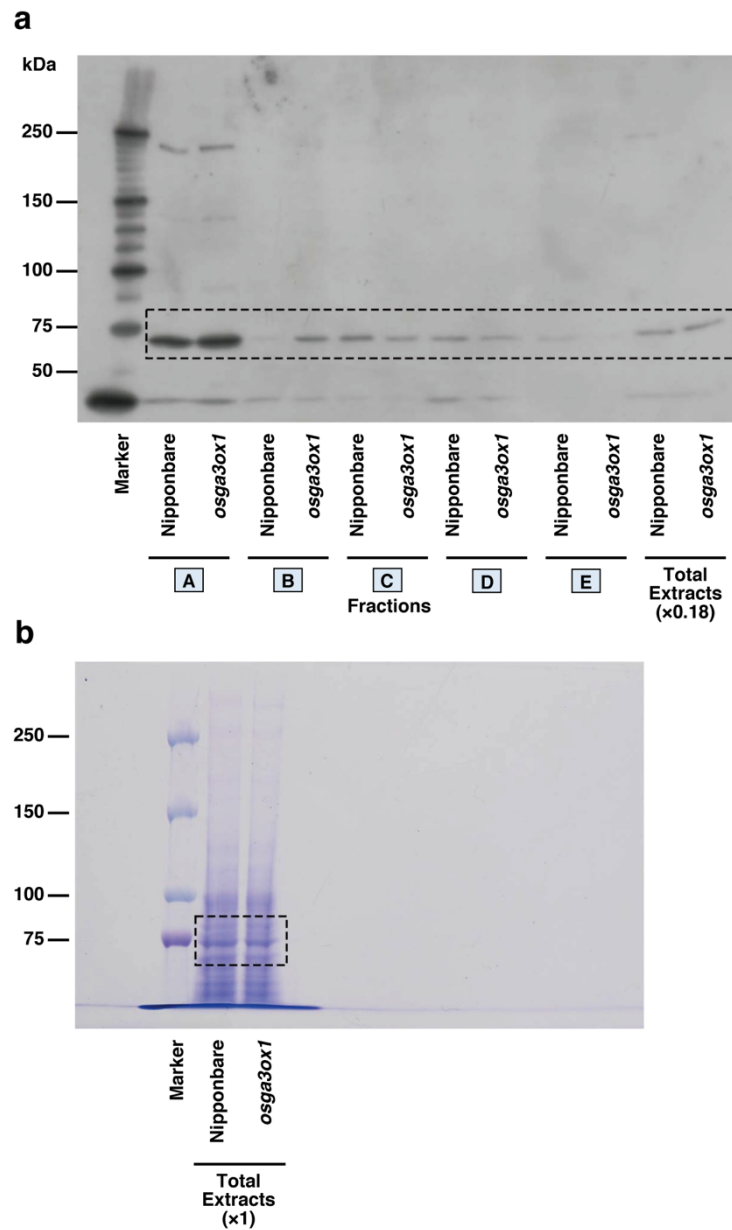


Supplementary Figure 11. Expression profiles of *GA3ox* orthologs in representative Poaceae plants. Tissue and developmental expression profile data of *GA3ox* orthologs in the Poaceae species *Triticum aestivum*, *Hordeum vulgare*, *Brachypodium distachyon*, *Sorghum bicolor*, and *Zea mays*. Expression profile data were obtained from the electronic fluorescent pictographs (eFP) browsers of the Brachypodium eFP Browser (*Brachypodium distachyon*) and Maize eFP Browser (*Zea mays*) (<http://bar.utoronto.ca>). Data for barley (*Hordeum vulgare*), wheat (*Triticum aestivum*), and sorghum (*Sorghum bicolor*) were obtained from PlaNet¹⁵ (<http://aranet.sbs.ntu.edu.sg/index.html>), Wheat Expression Browser^{16,17} (<http://www.wheat-expression.com>), and MOROKOSHI Sorghum transcriptome database¹⁸, respectively. Signals of anthers or corresponding to them are indicated.



Supplementary Figure 12. Schematic of complementation test and phenotypic analysis. a) Schematic of transformation of *GA3ox1* orthologs of *Oryza* into *osga3ox1* mutant. *GA3ox1* orthologs (green); *GA3ox1* neighbor genes (grey); intergenic region (black line); primer for PCR (red arrow) in the left side of the diagram. **b)** Quantification of the staining value of KI-stained pollen grains of

transgenic plants and vector control. Error bars, s.d. n = 300, one-way ANOVA with Tukey's multiple comparison test. Different letters denote significant differences ($p < 0.05$). **c**) Seed setting rate of transgenic plants described in panel **a** and vector control. n = 3 panicles for each line; statistical analysis as mentioned in panel **b**. Figure 5**d** and **e**, and Supplementary Figure 12**b** and **c** represent the same experiment performed with different independent lines. **d**) qRT-PCR expression analysis of *GA3ox1* in anthers and leaf blades of each transformant. Error bars, s.d. n = 3, one-way ANOVA with Tukey's multiple comparisons test. Different letters denote significant differences ($p < 0.05$). **e**) The vegetative phenotype of each transformant. Bar = 50 cm. **e** Endogenous GA levels in anthers of transformant. **f**) Evaluation of plant heights of each transformant. Error bars, s.d. n = 5, one-way ANOVA. n.s.; not significant ($p > 0.05$). **g**) Endogenous GA levels in the anthers of transgenic plants and vector control. n = 2 per panicle. The line numbers used in **b-g** correspond to each other.



Supplementary Figure 13. Uncropped images of western blot and the CBB-stained gel. Full images of western blot (**a**) and CBB staining (**b**) that were depicted in part in Figure 2k. Dashed line represents the cropped area.

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