

New Phytologist Supporting Information

Article title: The *osa-miR164* target *OsCUC1* functions redundantly with *OsCUC3* in controlling rice meristem/organ boundary specification

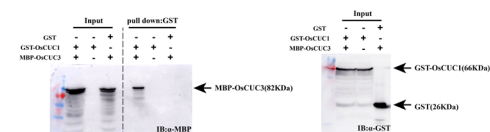
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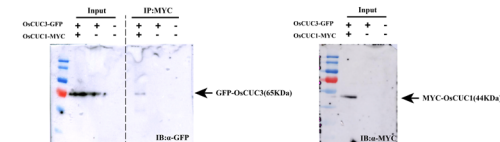
The following Supporting Information is available for this article:

Figure S1. The original blot images in this study.

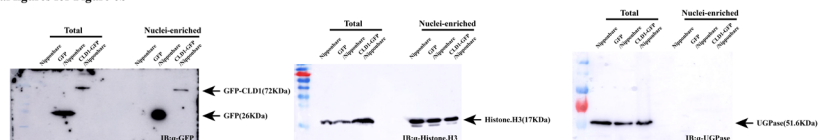
Original figures for Figure 4c



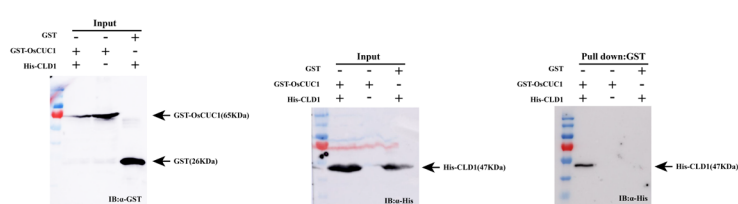
Original figures for Figure 4d



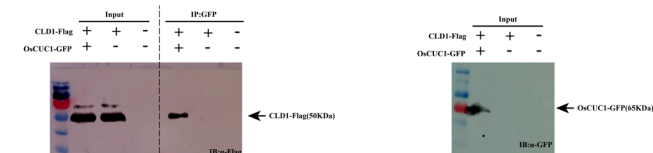
Original figures for Figure 6b



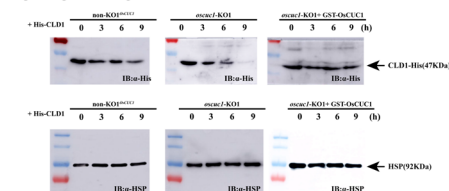
Original figures for Figure 6c



Original figures for Figure 6f



Original figures for Figure 7a



Original figures for Figure 7b

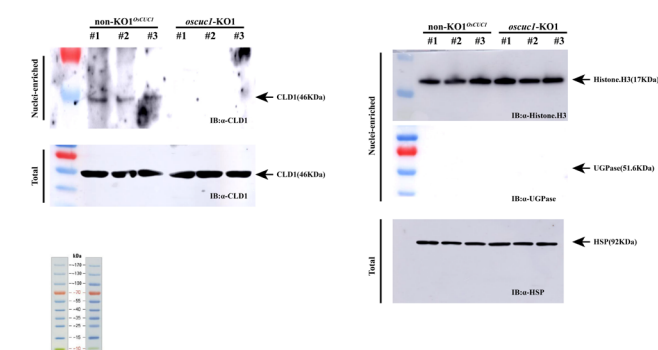


Fig S2. Sequence analysis of OsCUC1 and OsCUC3. (a) Sequence alignment of OsCUC1 and CUC1. (b) Sequence alignment of OsCUC3 and CUC3. The NAC domain is showed in the orange frame, the amino acid corresponding to *miR164* binding region (miR164BR) is showed in green frame. (c) Phylogenetic tree analysis of rice OsCUC1, OsCUC3 and their homologous from *Arabidopsis thaliana*, *Oryza sativa*, *Fragaria vesca*, *Solanum lycopersicum*, *Hordeum vulgare*, *Glycine max*, *Triticum aestivum*, *Rosa chinensis*, *Actinidia deliciosa*, *Citrus clementine*, *Vitis vinifera*, *Petunia hybrid* and *Populus trichocarpa*. Gene IDs are shown. The numbers indicate the bootstrap values calculated from 1000 replicates analyses.

a

		NAC domain	
OsCUC1	...NERCSVGLGCGGGGGRLDGLPPGFRFHPTDEELI	TYLLRKVVDGSENGRAIAEIDLNKCEPWELPEKAKAGEK	77
CUC1	...NDVFNAGFRREFDESINPPGFRFHPTDEELI	TYLLKKVLDSESCAASQVDLNKCEPWELPEKAKAGEK	74
CUC2	...NDIYYHYDVG...DSQV	LPPGFRFHPTDEELI	71
OsCUC1	EWFYFSLRDRKYPTGLRTNRATGAGYWKATGKDREIR	AR...TICALVGAKKTLVYFGRAPKGRITQWVYHEYRLDGLVA	155
CUC1	EWFYFSLRDRKYPTGLRTNRATEAGYWKATGKDREIR	SSR...TKSLLGAKKTLVYFGRAPKGEKSCWVYHEYRLDGLKFS	152
CUC2	EWFYFSLRDRKYPTGLRTNRATEAGYWKATGKDREIR	SSR...TICALVGAKKTLVYFGRAPKGEKSNWVYHEYRLDGLKFS	149
OsCUC1	VHFLSSSTRDEWVIRIRIGVFPVVRKRLGISGGGDTSCFSDST	ASVGGGGTSASALAPLADASTFAAAAA	235
CUC1	VHYISSSAKDEWVLCVKLRSGVSR	...ITNHS	189
CUC2	VHYISSSKDEWVIRVFCRTTLASTG	...AVSEGGCGGGATVSSTGTGPSKKTVPSTISNRYQEPSPSSVSLD	224
OsCUC1	AYGADSSN...YGGGGAGSAAATANLVGILVPCFS	...TITAHADAFGTGQINAPLAVEPPPPPAFAPS	304
CUC1	AYTGEFSS...AGSAAAPITNTAF	EHVSCFSNNSAAHTDASFEI...LPAPPPSLPPRCP	245
CUC2	PULDPTTLGYTDSSCSYDSRSTNTIVTASAI	EHVSCFSVYPTITTHALGDVNSISRLPPPLGDFDFPREVSRNVST	304
OsCUC1	...LRSLQENLQLPLSLSGCGAGYSSQPTSGGGAFHWCSGMDVKEGAVGRAPQMAVGP	COLDGAFWGF	373
CUC1	...RHVGDGVAFGQDLGLSSGQIDFAAAAFPNLPS	...LPPTMLPPPSPAFVYGGSPAVSVVPT	309
CUC2	QSNFNSFQENFNGFPVLCSSASTYTSAVNPSFQGGGGVSGNYSVLPATAEENESKYGULPAGDL	...CIVNT	375

b

		NAC domain	
OsCUC3	NGDAIYELMGEEMAAAAAAGEHCLPPGFRFHPTDEELVTFYLAAKVFNACCGGVDI	AEVDLNRCCEPWELPEAARAAGEK	80
CUC3	NTIAYEDVLSF...LAGEERNERCLPPGFRFHPTDEELITFYLAASKIFBGCTSC	ITITSEVDLNRCCEPWELPEAKAGER	76
OsCUC3	EWFYFSLRDRKYPTGLRTNRATGAGYWKATGKDREVM	AAAAGGALICAKKTLVYFGRAPRGEKTKWVLYHEYRLDGLDA	160
CUC3	EWFYFSLRDRKYPTGLRTNRATGAGYWKATGKDREVM	SGC...GGCLVGAKKTLVYFGRAPRKTWVLYHEYRLDGLDS	154
OsCUC3	A...ARRSLKEEWVIRIRIRIGVFGDOYSKIAM	...NKSASVYLPVSHH	202
CUC3	...PRHTCKEEWVIRVFNKIGDR	ENVGL...INQISYLENHSLS	194
OsCUC3	EPSSIFHLLP...VDFPNPSVPTFHDLPITSFHPPLIGESHANSNSNNGGVFPNPP	NTNSSDNITSCNGA	275
CUC3	TTHHHEALP...LLIIEPSNNTLTFPSLLYDDPEQVNNNTLHGSSGNTIDELKMLISPVVSLQNGI	FPSC	266
OsCUC3	...AAAAAAAFPSISCASTVIGKGGPPAQLGVNAGQEPPTVWDAMIQS	...GFIYEVGPPAVPRGA	340
CUC3	...NNNDEDDDFNLGVKTEQSSNC...NEIDVARDYLENPLEGEASVGLLGFSSSPGPI	ITNITLDSPCPLGFQ	333

c

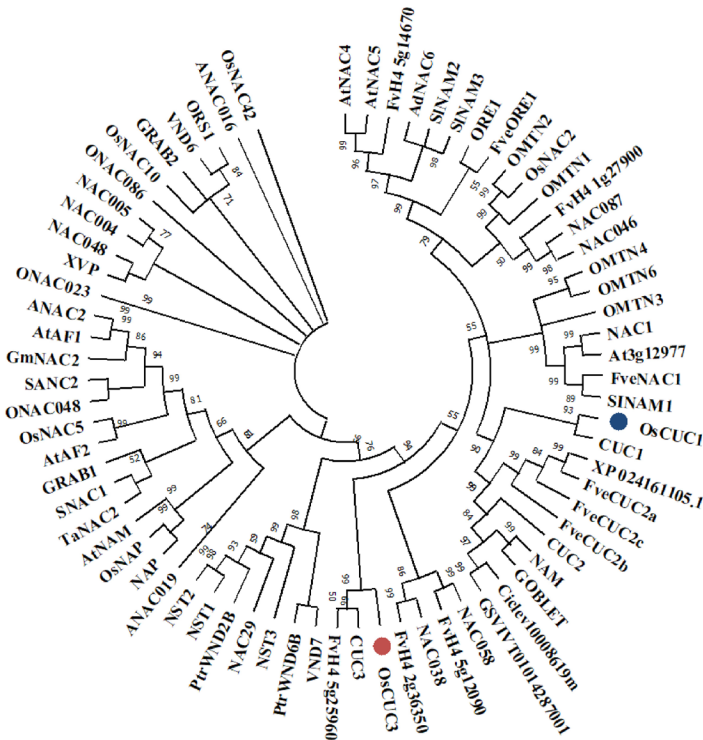


Fig S3. Loss-of-function of OsCUC1 and OsCUC3 generated by CRISPR/Cas9 in rice. (a) The *oscuc1*-KO1 harbors 1bp deletion leading to a frame-shift with premature transcription termination, the *oscuc1*-KO2 harbors 1bp insertion leading to a frame-shift with premature transcription termination. (b) Both *oscuc3*-KO1 and *oscuc3*-KO2 harbor 1 bp insertion at different sites of the coding region, result in a premature stop codon. The letters with colorful background indicate the identical amino acids with the wild type proteins.

a			
OsCUC1	MERCSVLGLGGGGGGGRLDGLPPGFRFHPTDEELI TYYLLRKVVDSFNGRAI AEI DL	60	
oscuc1-KO1	NERCSVLGLGGGGGGGRLDGLPPGFRFHPTDEELI TYYLLRKVVDSFNGRAI AEI DL	60	
oscuc1-KO2	NERCSVSGAGRVGRGAAAGRRRAAGVPVPPDGRGADHLLPAAEGGRELQRARHRGRDP	60	
OsCUC1	NKCEPWELPEKAKYGEKEWYFYSLRDRKYPTGLRTNRATGAGYWKATGKDREI RS ART GA	120	
oscuc1-KO1	NKCEPWELPEKAKYGEKEWYFYSLRDRKYPT . . . DSAPTAPRAPATGRP. PARTARS..	113	
oscuc1-KO2	EQVRAVGAAGEGQDGGEGVLLQPPRPQVPHG. . TPHQPRHGRRLLEGHRQGPDPQR. .	116	
OsCUC1	LVGMKKTLVFYRGRAPKQKQTQWVMEYRLDGTAYHF LSSSTRDEWVI ARI FTKPGVFP	180	
oscuc1-KO1		113	
oscuc1-KO2		116	
OsCUC1	VVRKGRLGI SGGGGDTS CFSDSTSASVGGGGGTSASSALRAPLAEASLFAAAAAPAVDGA	240	
oscuc1-KO1	 AAPAPAPSSA	123	
oscuc1-KO2	 PHRRPRRHEE	126	
OsCUC1	DS S NYGGGGGAGS ATATANLVTGLELVP CFSTTAHMDASFGTGQYNPAPLAVEPPPPPPA	300	
oscuc1-KO1		123	
oscuc1-KO2	DPRLPRPRPQGPEDPVG. HA	146	
OsCUC1	FFPSLRS LQENLQLPLFLS GGMQAGVSS QPLS GGGAFHWQS GMDVKVE GAVGRAPPQNAV	360	
oscuc1-KO1		123	
oscuc1-KO2	RVPPRRHLRLPLPLLHPGG.	166	
OsCUC1	GPQLDGAF AVGF	373	
oscuc1-KO1		123	
oscuc1-KO2		166	
b			
OsCUC3	MGDALWENLGEENAAAAAAGEHGLPPGFRFHPTDEELVTF YLAAKVFNACC GGVDI AE	60	
oscuc3-KO1	MGDALWENLGEENAAAAAAGEHGLPPGFRFHPTDEELVTF YLAAKVFNACC GGVDI AE	60	
oscuc3-KO2	MGDALWENLGEENAAAAAAGE TRAAAGVQVPPHRRGARHLLPRRQGVQRRVLRRRGRHG	60	
OsCUC3	VDLNRCEPWELPEAARMGEKEWYFYSLRDRKYPTGLRTNRATGAGYWKATGKDRE VVAAA	120	
oscuc3-KO1	VDL. KPVRVAVG	70	
oscuc3-KO2	GGP. EPVRVAVG	70	
OsCUC3	AAGGALI GAKKTLV FYKGRAPRGEKTKWLHE YRLDGDFAAARRS TKEEWVI CRI FHKVG	180	
oscuc3-KO1	AAGGEDDGGEGVLLQP. . . PRPQVPDGAHQPRHRRRLLEGHRQGGGGRRRRRRRRAH	127	
oscuc3-KO2	AAGGEDDGGEGVLLQP. . . PRPQVPDGAHQPRHRRRLLEGHRQGGGGRRRRRRRRAH	127	
OsCUC3	DQYSKLMMKSPASYYLPVSHHHPSSI F HDLPPVPFPNPSLVPFHHDLPSTFHPPLLQHS	240	
oscuc3-KO1	RHEEDARLLQGPR. . . PARRED. QVGPPRVPPR	156	
oscuc3-KO2	RHEEDARLLQGPR. . . PARRED. QVGPPRVPPR	156	
OsCUC3	HANSKNS S S NNGGF VFPNEPNTTNS SDNHI SCNGAMAAAAAFAF SFS CAS TVTGKGGPP	300	
oscuc3-KO1	RRLRRRS PLHQGGNGDLQDLS QGRRSVQQADDD. EEP	192	
oscuc3-KO2	RRLRRRS PLHQGGNGDLQDLS QGRRSVQQADDD. EEP	192	
OsCUC3	AQLGVNAGQQEPPPTWMDAYLQHS GF I YEMGPPAVPRGA	340	
oscuc3-KO1	SQLLP. . . PSEPPPPQHLPP.	209	
oscuc3-KO2	SQLLP. . . PSEPPPPQHLPP.	210	

Fig S4. Boundary specification defects in vegetative growing stage of the rice *oscuc1*-KO1 mutant. (a) and (b) Histologic slides (cross section) of leaf blade (the basal of the leaf blade) for *oscuc1*-KO1 and its non-KO control. (c) and (d) Histologic slides (cross section) of leaf sheath (1 cm below the flag leaf) for *oscuc1*-KO1 and its non-KO control. Scale bars = 500 μ m.

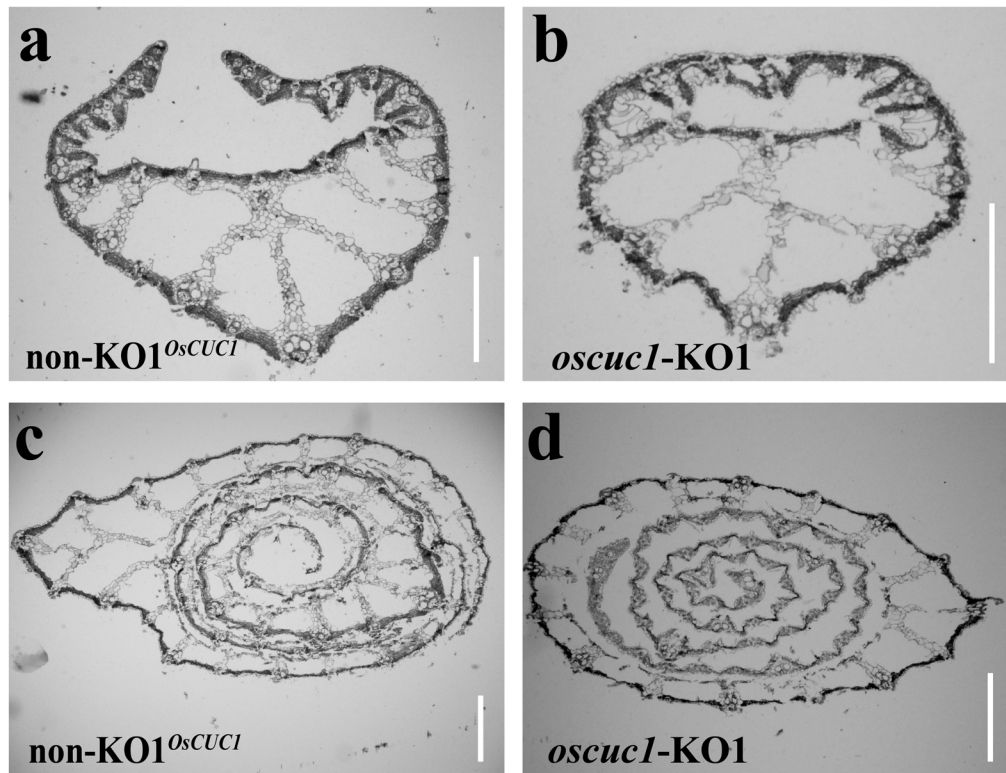


Fig S5. Pollen defects of the rice *oscuc1*-KO1 mutant. (a) and (b) Pollen viability test for non-KO^{OsCUC1} and *oscuc1*-KO1 plant, represents the pollen activity test for 3 biological replicates of each line respectively. Scale bar = 500 μ m.

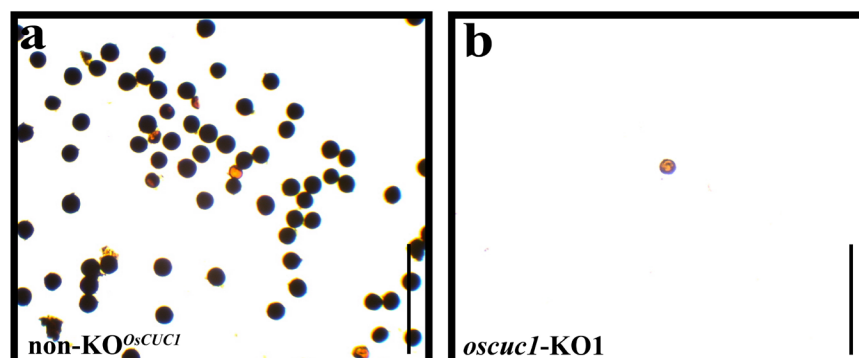


Fig S6. Phenotypes of heterozygous mutants of *OsCUC1* and *OsCUC3* in rice. (a) and (b) The panicle of T₀ non-KO^{*OsCUC1*} and *oscuc1*-KO1. (c) Self-pollinated florets of T₀ *oscuc1*-KO1. (d) Cross-pollinated florets which pollen from Nipponbare was used to pollinate the gynoecia of *oscuc1*-KO1. (e) Detail of the pane in (d). (f) and (g) F₂ generation of non-KO^{*OsCUC1*}, *oscuc1*-KO1/+ and *oscuc1*-KO1 in seedling stage and heading stage, all of these plants were derived from a F₁ *oscuc1*-KO1/+ plant. (h) and (i) non-KO^{*OsCUC3*}, *oscuc3*-KO1/+ and *oscuc3*-KO1 in seedling stage and heading stage, all of these plants were derived from a *oscuc3*-KO1/+ plant.

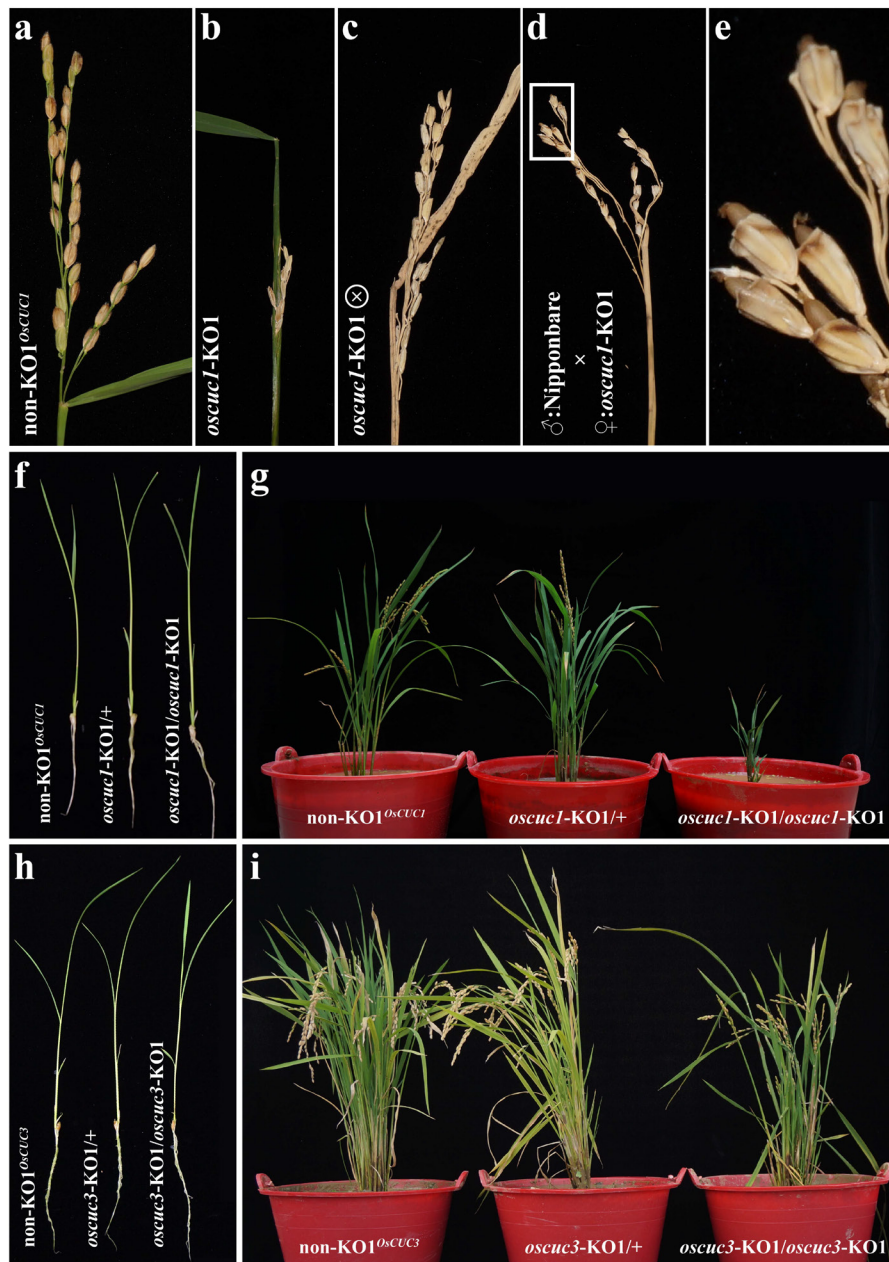


Fig S7. Dimerization of the Arabidopsis CUC proteins. (a) BiFC assay indicated that the CUC1, CUC2 and CUC3 do not form heterodimers or homodimers. Interaction between AP1 and SEP3 was used as positive control. (b) The schematic of constructing *mCUC1* and *mCUC2*. The red letters indicate mutant nucleotides introduced into the *miR164* target site in the *mCUC1* or *mCUC2* construction, which interrupts the *miR164* target site without changing the amino acid residues. Watson-Crick base pairing between the mRNA and *miR164* is indicated by black lines. Mismatches and G:U wobbles are indicated by a star or equals sign, respectively. (c) BiFC assay indicated the homodimerization of mCUC1 and mCUC2, and the heterodimerization of mCUC1-mCUC2, mCUC1-CUC3 and mCUC2-CUC3. Co-expression of GFP_C-mCUC1 plus GFP_N or GFP_C plus GFP_N-mCUC1 or GFP_C-mCUC2 plus GFP_N or GFP_C plus GFP_N-mCUC2 or GFP_C-CUC3 plus GFP_N or GFP_C plus GFP_N-CUC3 were used as negative controls. Scale bars = 5 μ m.

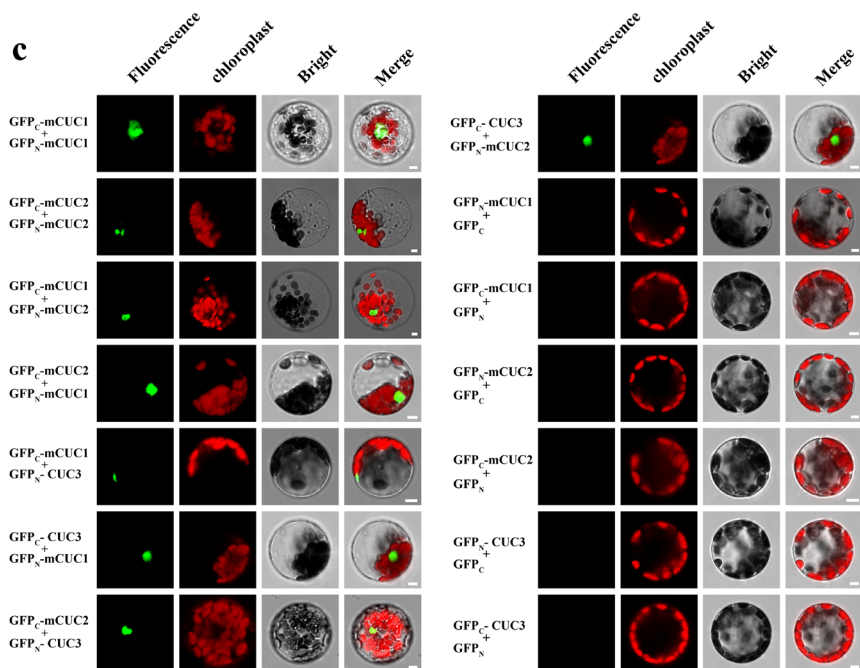
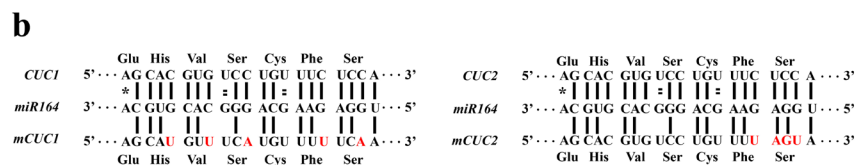
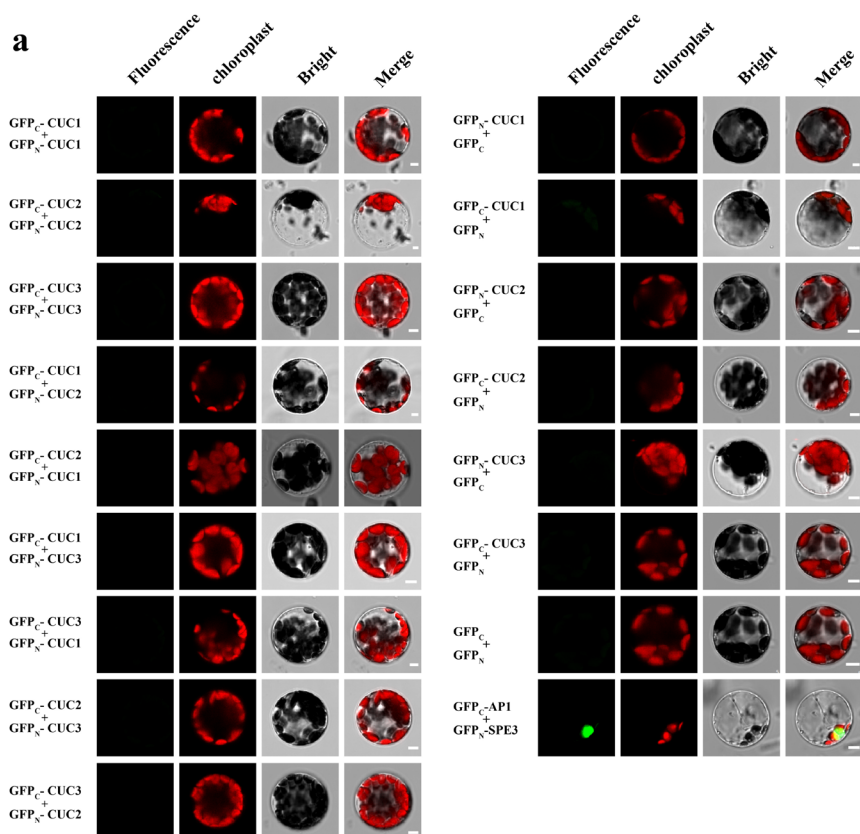


Fig S8. The development of rice *oscuc1 oscuc3* homozygous double mutants is arrested in the seedling stage. (a) to (d) Histologic slides (vertical section) of SAM for wild type (Nipponbare), *oscuc1*, *oscuc3* and *oscuc1 oscuc3*. Scale bars = 50 μ m. (e) to (h) Appearance of *oscuc1/+ oscuc3/oscuc3* and three *oscuc1 oscuc3* homozygous double mutants in 15 DAG (day after germination). (i) to (l) Appearance of the corresponding plants in 36 DAG (day after germination). Scale bars = 1 cm.

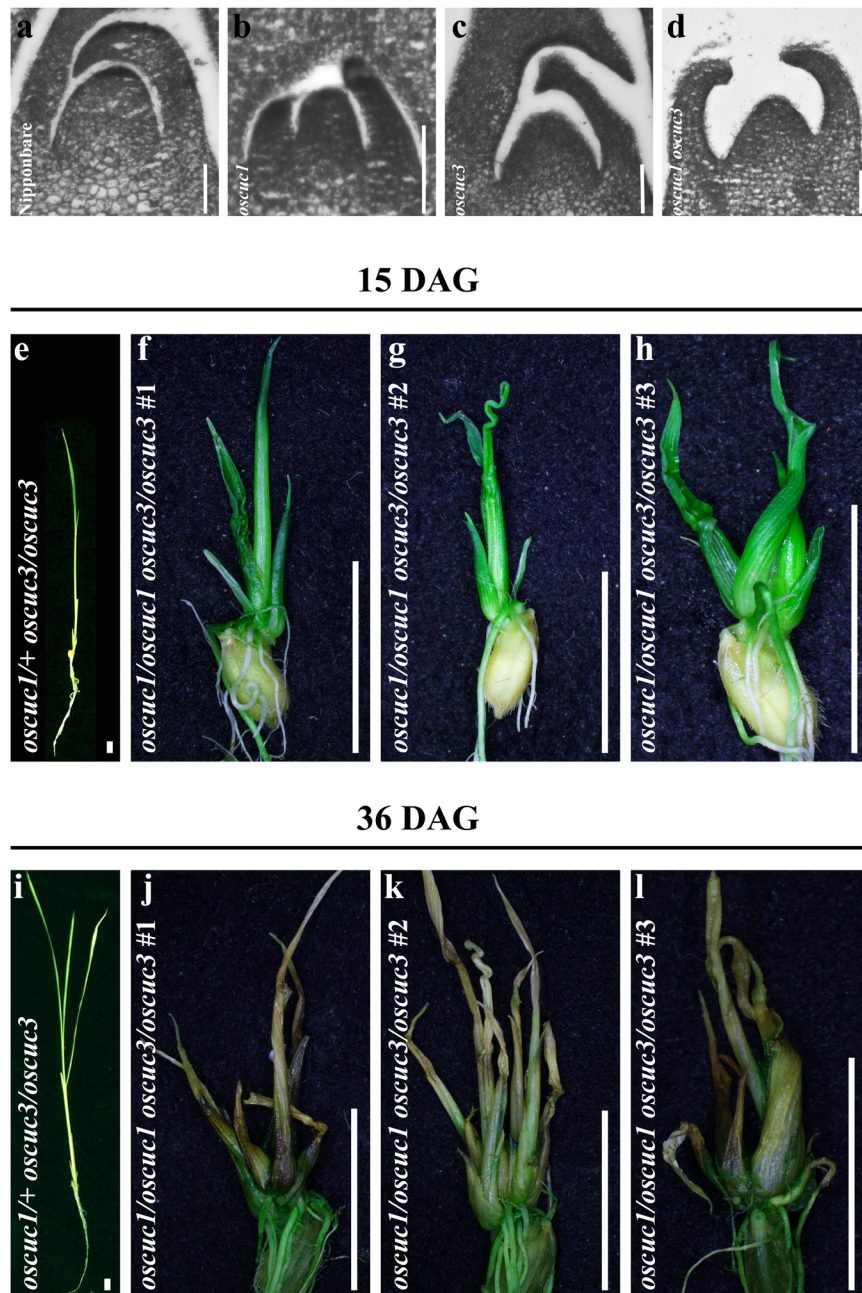


Fig S9. Transcript evidence and expression pattern of rice *osa-miR64c*. (a) RT-PCR with specific primers for *pre-osa-miR164c* using the Nipponbare cDNA as the templates. The product from reverse transcription without reverse transcriptase was used as negative control in the RT-PCR. Three biological replicates were used in this assay. (b) The relative expression level of *pre-osa-miR164c* in seedling (3 days), leaf, root, UBI (unelongated basal internode), leaf sheath, bract, flag leaf, YP 0.5 cm (young panicle 0.5 cm), YP 2 cm (young panicle 2 cm), YP 8 cm (young panicle 8 cm), stamen, pistil, palea and lemma. Values are shown as means $\pm$ s.d. (n = 3).

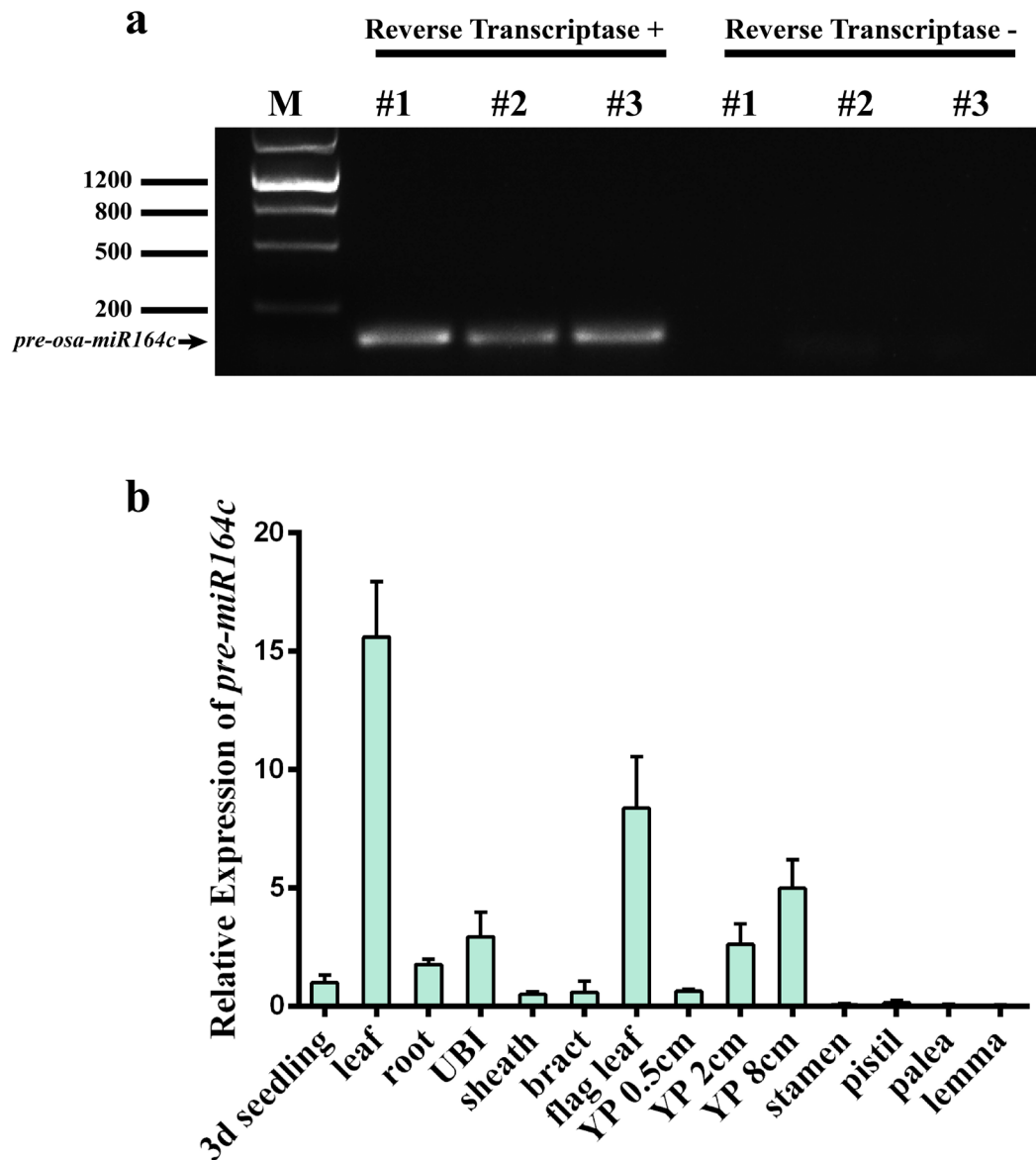


Fig S10. The expression patterns of the other five *osa-miR164* targets in rice. (a) to (e) The relative expression level of *OMTN1*, *OMTN2*, *OMTN3*, *OMTN4* and *OMTN6* in leaf, root, flag leaf, pistil, stamen, palea and lemma for two individual *OE-osa-miR164c* lines and the empty-vector control line (EVC). Values are shown as means $\pm$ s.d. ($n = 3$).

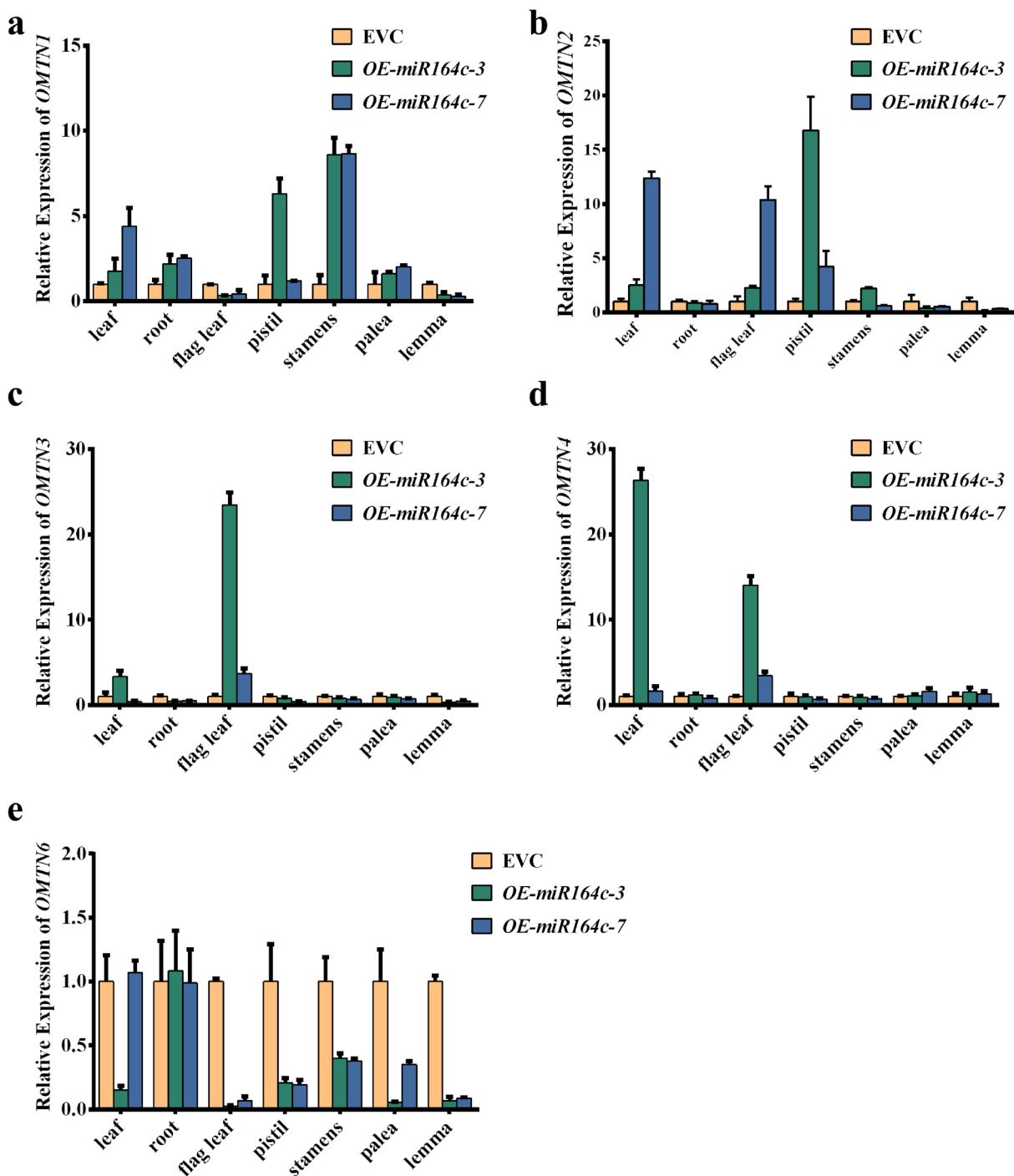


Fig S11. Knocking out *OMTN4* or *OMTN6* dose not lead to defects either in boundary specification or in leaf development in rice.^{[1][SEP]} (a) Schematic diagram of *OMTN4* gene structure and the CRISPR/Cas9 target site. The PAM site is underline. The mutant site is shown in red. (b) The phenotype of the *omtn4*-KO1 line and non-KO^{*OMTN4*} control line. The genetic background of both lines is Nipponbare. (c) The phenotype of the *omtn4*^{ZH11} and control line Zhonghua11 (ZH11). (d) Schematic diagram of *OMTN6* gene structure and the CRISPR/Cas9 target site. The PAM site is underline. The mutant site is shown in red. (e) The phenotype of the *omtn6*-KO1 line and non-KO^{*OMTN6*} control line. The genetic background of both lines is Nipponbare. (f) The phenotype of the *omtn6*^{ZH11} and control line Zhonghua11.

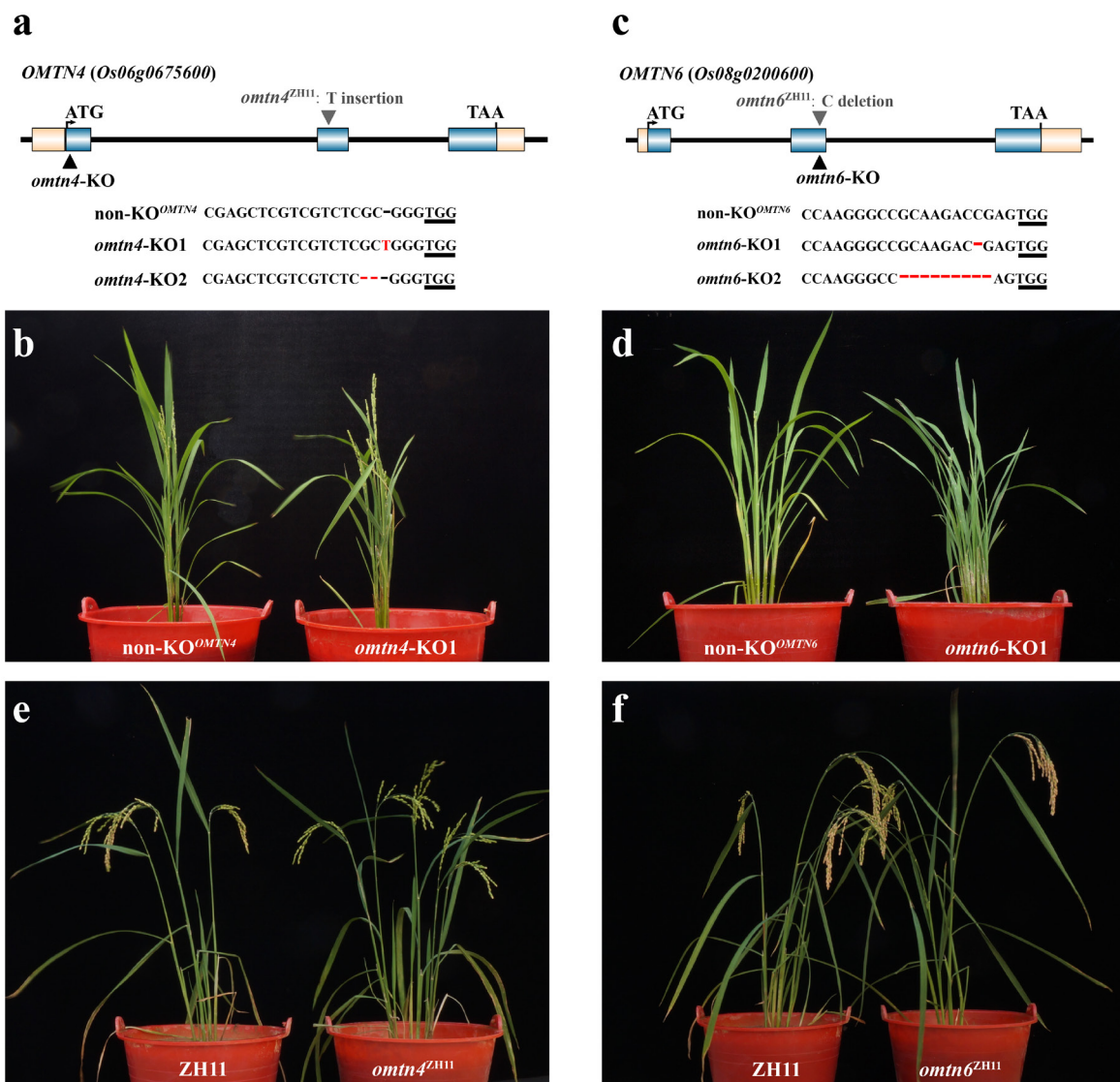


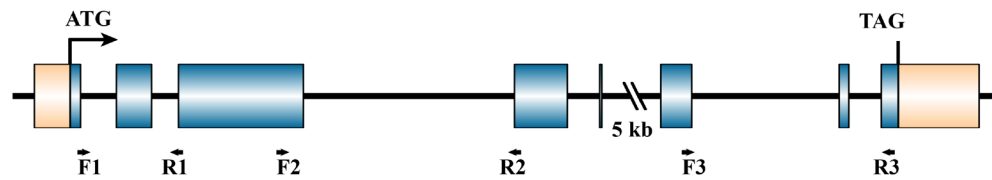
Fig S12. The *CLD1* expression level does not significantly change in the rice *oscuc1* mutant.

(a) Structure of *CLD1* gene. The three pairs of arrows indicated the three pairs of primers using for qRT-PCR. (b) The relative expression level of *CLD1* evaluated by three pairs of primers.

Values are shown as means $\pm$ s.d. (n = 3).

a

CLD1 (Os07g0102300)



b

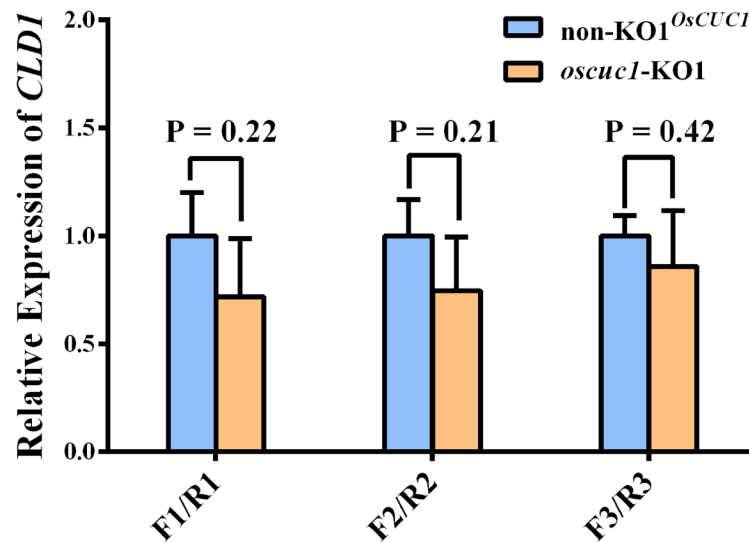


Fig S13. CLD1 does not interact with OsCUC3, OMTN4 or OMTN6 in rice. (a) BiFC assay indicated that CLD1 does not interact with OsCUC3, OMTN4 or OMTN6. Co-expression of GFP_C-OsCUC1 plus GFP_N-CLD1 was used as positive control. Scale bars = 5 μ m. (b) Y2H assay indicated that CLD1 does not interact with OsCUC3, OMTN4 or OMTN6. The combination of BK-53 plus AD-T was used as a positive control, while BK-LAM plus AD-T or BK plus AD were used as negative controls.

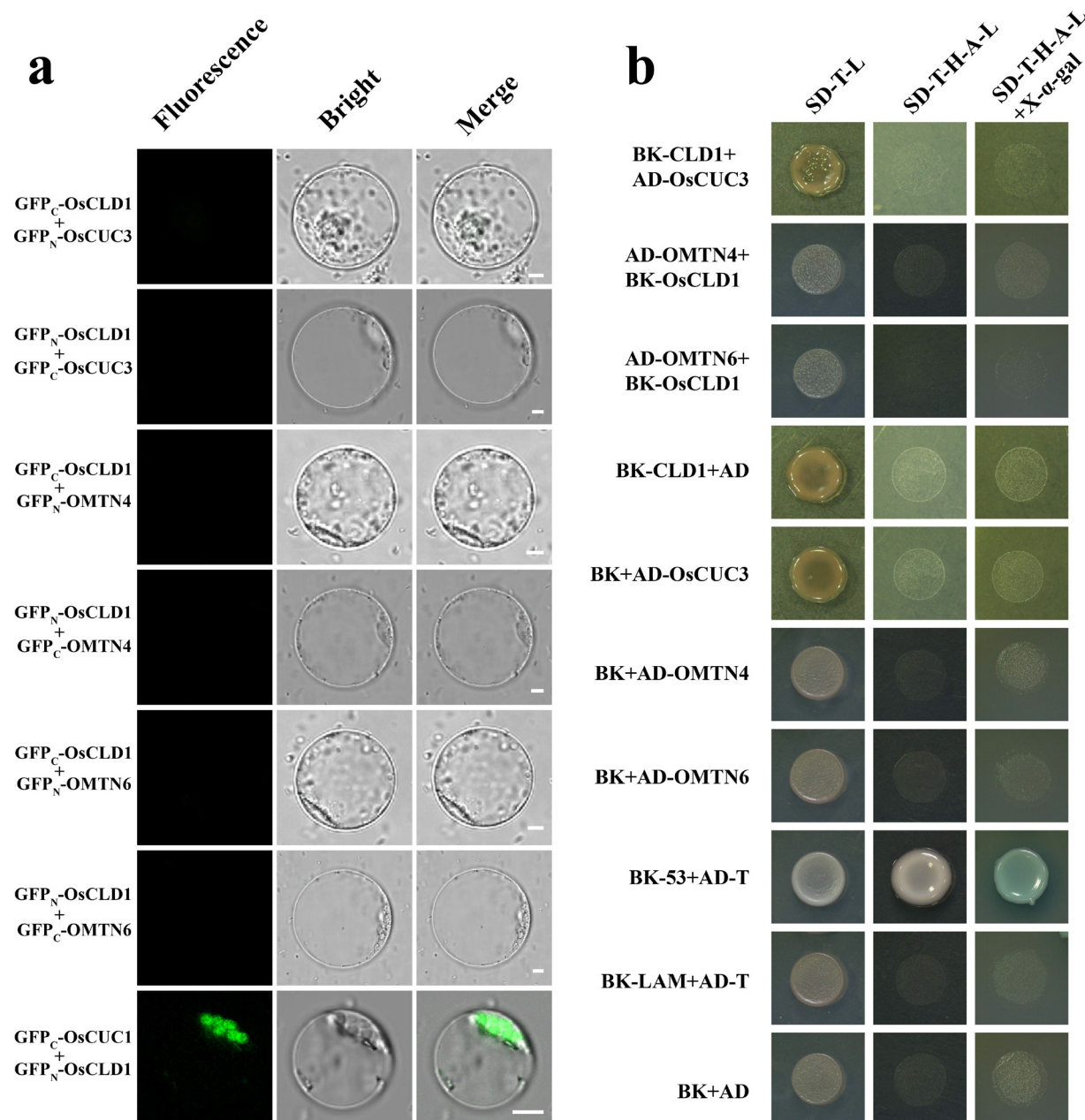


Table S1. The accession numbers for the proteins listed in phylogenetic tree

Species	Protein name	Accession numbers
<i>Arabidopsis thaliana</i>	CUC1	NP_188135
	CUC2	NP_200206
	CUC3	XP_015648828
	NAC004	NP_171726
	NAC005	NP_171727
	NAC048	AAN38683
	VND6	ABE02409
	VND7	OAP19653
	NAP	NP_564966
	XVP	NP_171725
	ANAC2	NP_171677
	ORE1	NP_198777
	NAC1	AAO64808
	AtNAC4	AAP21227
	NAC058	NP_188469
	NAC038	NP_850054
	NAC087	NP_974800
	NAC046	NP_187056
	AtNAC5	NP_001331908
	ORS1	OAP09180
	At3g12977	NP_001327438
	ANAC016	ABO38757
	ANAC019	NP_175697
	NST1	NP_182200
	NST2	OAP06131
	NST3	NP_174554
	AtNAM	NP_175696
	AtAF1	NP_171677
	AtAF2	NP_680161

<i>Oryza sativa</i>	OsCUC1	XP_015642575
	OsCUC3	BAC57407
	OMTN1	XP_015623389
	OMTN2	XP_015633922
	OMTN3	XP_015620576
	OMTN4	XP_025881778
	OMTN6	XP_015648318
	ONAC086	XP_015638590
	NAC29	AEO53047.1
	OsNAP	BAH01634
	OsNAC2	BAG88085.1
	ONAC023	BAG98022.1
	ONAC048	BAG90892.1
	OsNAC5	AB028184.1
	SNAC1	BAG90542.1
	OsNAC10	BAG91345.1
	OsNAC42	BAG90930.1
	SNAC2	BAG90892.1
<i>Fragaria vesca</i>	FveCUC2a	XP_004291499
	FveCUC2b	FvH4_6g06070
	FveCUC2c	FvH4_3g03540
	FvH4_5g25960	XP_004301620
	FvH4_5g12090	XP_004300523
	FvH4_2g36350	XP_004291275
	FvH4_1g27900	XP_004309786
	FvH4_5g14670	XP_004299340
<i>Fragaria vesca</i>	FveORE1	XP_004304286
	FveNAC1	XP_004294208
<i>Solanum lycopersicum</i>	GOBLET	ACL14371
	SINAM2	solyc03g115850
	SINAM3	solyc06g069710

<i>Solanum lycopersicum</i>	SINAM1	solyc07g066330
<i>Hordeum vulgare</i>	GRAB1	KAE8820591
	GRAB2	KAE8816523
<i>Glycine max</i>	GmNAC2	AAY46122
<i>Triticum aestivum</i>	TaNAC2	AAU08786
<i>Rosa chinensis</i>	XP_024161105.1	XP_024161105
<i>Actinidia deliciosa</i>	AdNAC6	AZL19352
<i>Citrus clementina</i>	Ciclev10008619m	ESR65017
<i>Vitis vinifera</i>	GSVIVT01014287001	CBI20259
<i>Petunia hybrida</i>	NAM	X92205
<i>Populus trichocarpa</i>	PttrWND2B	XP_024450691
	PttrWND6B	ADR00341

Table S2. The primer sequences using in this study.

Primer sequences for generating DNA constructs			
Experiment	Construct	Primer name	Sequence (5'→3')
overexpressed <i>osa-miR164c</i>	<i>pUbi::osa-miR164c</i>	OE-miR164cF	AACTGCAGTTGATGCTACTGTAGCCATC
		OE-miR164cR	GGACTAGTTCCTATCATTGATTCATTG
GUS assay	<i>pOsCUC1::GUS</i>	OsCUC1pgus-F	CCCAAGCTTTGCTCTCGACCTGTAGATTCC
		OsCUC1pgus-R	GCTCTAGACCAGCTGCTGCAACAAGTAAC
	<i>pOsCUC1::OsCUC1-GUS</i>	pro-cds-gus-F	ACGCGTCGACTGCTCTCGACCTGTAGATTCC
		pro-cds-gus-R	GCTCTAGAGAAGCCCCATGCAAAGGCGCCG
Yeast two-hybrid assay	<i>pOsCUC1-mOsCUC1-GUS</i>	mOsCUC1-R	CGAAAAACATGGGACCAGTTCAAGGCCGGTGACCAAGTTGGC
		mOsCUC1-F	ACTGGTCCCAGTTTTCGACCACAGCCCACATGGATGCCTC
	<i>pGADT7-OsCUC1</i>	OsCUC1-AD-F	CGGAATTCATGGAGCGGTGCAGCGTGC
		OsCUC1-AD-R	ACGCGTCGACCTAGAAGCCCCATGCAAAG
	<i>pGADT7-OsCUC3</i>	OsCUC3-AD-F	CGGAATTCATGCATCATCACTCGGCCAC
		OsCUC3-AD-R	CGGGATCCTCATGCGCCCCTGGGCACTG
	<i>pGADT7-OMTN4</i>	OMTN4-F	GGAATTCATATGATGAGCGGGATGAATTCGCTGAGC
		OMTN4-R	CCCTCGAGTCAACTGAGTGAGTTCCACATTTGTGTG
	<i>pGADT7-OMTN6</i>	OMTN6-F	CGGAATTCATGAGTTTCATAGGCATGGTGG
		OMTN6-R	CGGGATCCTTATTGGGGGTCCACATCTGTGAC
Transactivation assay	<i>pGBKT7-CLD1</i>	CLD1-BD-F	GGAATTCATATGATGGGGATGGCCCCCGCCGGTGC
		CLD1-BD-R	CGGAATTCCTAGACTTGTAGCCAAATAGCAACAAC
	<i>pGBKT7-OsCUC1</i>	OsCUC1-BD-F	CGGAATTCATGGAGCGGTGCAGCGTGC
		OsCUC1-BD-R	CGGGATCCCTAGAAGCCCCATGCAAAG
	<i>pGBKT7-OsCUC3</i>	OsCUC3-BD-F	CGGAATTCATGCATCATCACTCGGCCAC
		OsCUC3-BD-R	CGGGATCCTCATGCGCCCCTGGGCACTG
BiFC assay	<i>p35S::CLD1-Vc/Vn</i>	CLD1-Vc/Vn-F	CGGAATTCATGGGGATGGCCCCCGCCGGTGC
		CLD1-Vc/Vn -R	GGAATTCATATGGACTTGTAGCCAAATAGCAAC
	<i>p35S::OsCUC1-Vc/Vn</i>	OsCUC1-Vc/Vn-F	CGGAATTCATGGAGCGGTGCAGCGTGTGGGG
		OsCUC1-Vc/Vn -R	GGAATTCATATGGAAGCCCCATGCAAAGGCGCCG
	<i>p35S::OsCUC3-Vc/Vn</i>	OsCUC3-Vc/Vn-F	CGGGATCCATGCATCATCACTCGGCCACCATGGG
		OsCUC1-Vc/Vn-R	CGGAATTCAGTGCAGCCCCTGGGCACTGCAGGTG
	<i>p35S::OMTN4-Vc/Vn</i>	OMTN4-Vc/Vn-F	GGGGACTCTAGGATCATGAGCGGGATGAATTCGCTGAGC
		OMTN4-Vc/Vn-F	CTCCATGATCGAATTACTGAGTGAGTTCCACATTTGTGTG
	<i>p35S::OMTN6-Vc/Vn</i>	OMTN6-Vc/Vn-F	GGGGACTCTAGGATCATGAGTTTCATAGGCATGGTGG
		OMTN6-Vc/Vn-F	CTCCATGATCGAATTTTGGGGGTCCACATCTGTGAC
	<i>p35S::AtCUC1-Vc/Vn</i>	<i>AtCUC1-Vc/Vn-F</i>	GGGGACTCTAGGATCATGGATGTTGATGTGTTAACGGT
		<i>AtCUC1-Vc/Vn-R</i>	CTCCATGATCGAATTGAGAGTAAACGGCCACACAC
	<i>p35S::AtCUC2-Vc/Vn</i>	<i>AtCUC2-Vc/Vn-F</i>	GGGGACTCTAGGATCATGGACATTCCGTATTACCAC
		<i>AtCUC2-Vc/Vn-R</i>	CTCCATGATCGAATTGTAGTTCCAAATACAGTCAAGT
	<i>p35S::AtCUC3-Vc/Vn</i>	<i>AtCUC3-Vc/Vn-F</i>	GGGGACTCTAGGATCATGATGCTTGCGGTGGAAGAT
		<i>AtCUC3-Vc/Vn-R</i>	CTCCATGATCGAATTCAGCTGGAATCCTAAAGGACAT

Subcellular localization assay	<i>p35S::OsCUC1-eGFP</i>	eGFP-OsCUC1-F eGFP-OsCUC1-R	CCCTCGAGATGGAGCGGTGCAGCGTGC ACGCGTCGACGAAGCCCCATGCAAAGGCG
	<i>p35S::OsCUC3-eGFP</i>	eGFP-OsCUC1-F eGFP-OsCUC1-R	CCCTCGAGATGCATCATCACTCGGCCACCATGGG ACGCGTCGACTGCGCCCCTGGGCACTGCAGGTG
	<i>p35S::CLD1-eGFP</i>	eGFP-CLD1-F eGFP-CLD1-R	GCTCTAGACATGGGGATGGCCCCGCCGGTGC TCCCCCGGGCTAGACTTGTAGCCAAATAGCAACAAC
pull-down assay	<i>OsCUC1/pGEX-6p-1</i>	GST-OsCUC1 -F GST-OsCUC1 -R	GGGATCCCCGGAATTCATGGAGCGGTGCAGCGTGCT GGCCGCTCGAGTCGACCTAGAAGCCCCATGCAAAGGCG
	<i>CLD1/pRSFDuet-His</i>	His-CLD1 -F His-CLD1 -R	CCAGGGATCCGAATTCGAGGAGGAGGAGGACGGCAGCGG ATGCGGCCGCAAGCTTCTATCTGTTCTGGTTGACACCATT
	<i>OsCUC3/pRSFDuet-MBP</i>	MBP-OsCUC3 -F MBP-OsCUC3- R	CCAGGGATCCGAATTCATGCATCATCACTCGGCCACCAT ATGCGGCCGCAAGCTTTCATGCGCCCCCTGGGCACTGCAGG
	<i>pCMVTNTTM-GST-OsCUC1</i>	OsCUC1-pTnT-F OsCUC1-pTnT-R	TTGCACTCGAGAATTCATGTCCCCTATACTAGGTTATTGGA GGCCGCCCGGGTCGACCTAGAAGCCCCATGCAAAGGCG
	<i>pCMVTNTTM-MBP-OsCUC3</i>	OsCUC3-pTnT-F OsCUC3-pTnT-R	TTGCACTCGAGAATTCATGAAATCGAAGAAGGTAACTGGTA GGCCGCCCGGGTCGACTCATGCGCCCCTGGGCACTGCAGG
	<i>pCMVTNTTM-His-CLD1</i>	CLD1-pTnT-F CLD1-pTnT-R	TTGCACTCGAGAATTCATGGGCAGCAGCCATCACCATCATC GGCCGCCCGGGTCGACCTAGACTTGTAGCCAAATAGCAACAACAAG

Primer sequences for real-time PCR analyses

Gene ID	Gene Name	Sequence (5'→3')
<i>LOC_Os06g23650</i>	<i>OsCUC1</i>	Forward primer: TCCTCCACCCGGGATGAGTG Reverse primer: CGAGAAGCACGAGGTGTCGC
<i>LOC_Os08g40030</i>	<i>OsCUC3</i>	Forward primer: CGCTCCACCAAGGAGGAATGG Reverse primer: GACCGGAGGAAGGTCATGGAAG
<i>LOC_Os02g36880</i>	<i>OMTN1</i>	Forward primer: AGGACACCGGCCTCACGT Reverse primer: GATCCATGCGTTGCTGCC
<i>LOC_Os04g38720</i>	<i>OMTN2</i>	Forward primer: TCGCGATCGAGGCATCTAC Reverse primer: CCCAAATCACCAAATAATCTACACCTA
<i>LOC_Os04g38720</i>	<i>OMTN3</i>	Forward primer: TGCCTGAATTTGGCAGCTT Reverse primer: ACACCTCACCCCCAATGGA
<i>LOC_Os06g46270</i>	<i>OMTN4</i>	Forward primer: ACAATATGGGTAGGGCGATCAA Reverse primer: TCCCTCTTCACATTGCCTTCA
<i>LOC_Os08g10080</i>	<i>OMTN6</i>	Forward primer: GGTCGGGAGCTACGAGCAA Reverse primer: TGATGGAGCCCTTCATTGG

qRT-PCR primers for *osa-miR164*

Primer name	Sequence (5'→3')
Universal Reverse primer	GTGCAGGGTCCGAGGT
RT primer for <i>U6</i>	GTTGGCTCTGGTGCAGGGTCCGAGGTATTCGCACCAGAGCCAACAA ATT
Forward primer for <i>U6</i>	CGCACAAATCGAGAAATGGTCC
RT primer for <i>osa-miR164c</i>	GTTGGCTCTGGTGCAGGGTCCGAGGTATTCGCACCAGAGCCAACCTG CACG
Forward primer for <i>osa-miR164c</i>	CGGCGGTGGAGAAGCAGGGTA

Table S3. Percentages of aberrant florets of *oscuc1*-KO and *oscuc3*-KO plants.

Line	Reduction of stamens	Fusion of filaments
<i>oscuc1</i> -KO1	96%	34%
non-KO1 ^{<i>OsCUC1</i>}	0%	0%
<i>oscuc1</i> -KO2	100%	32%
non-KO2 ^{<i>OsCUC1</i>}	0%	0%
<i>oscuc3</i> -KO1	92%	100%
non-KO1 ^{<i>OsCUC3</i>}	0%	0%
<i>oscuc3</i> -KO2	6%	52%
non-KO2 ^{<i>OsCUC3</i>}	0%	0%

For each line, 100 florets were used for the statistic analysis.

Table S4. Percentages of aberrant florets of *omtn4*-KO1 and *omtn6*-KO1 plants.

Line	Reduction of stamens	Fusion of filaments
<i>omtn4</i> -KO1	4%	5%
non-KO ^{<i>OMTN4</i>}	0%	0%
<i>omtn6</i> -KO1	2%	4%
non-KO ^{<i>OMTN6</i>}	0%	0%

For each line, 100 florets were used for the statistic analysis.