

Supplementary Information

Rice APC/C^{TE} controls tillering through mediating the degradation of MONOCULM 1

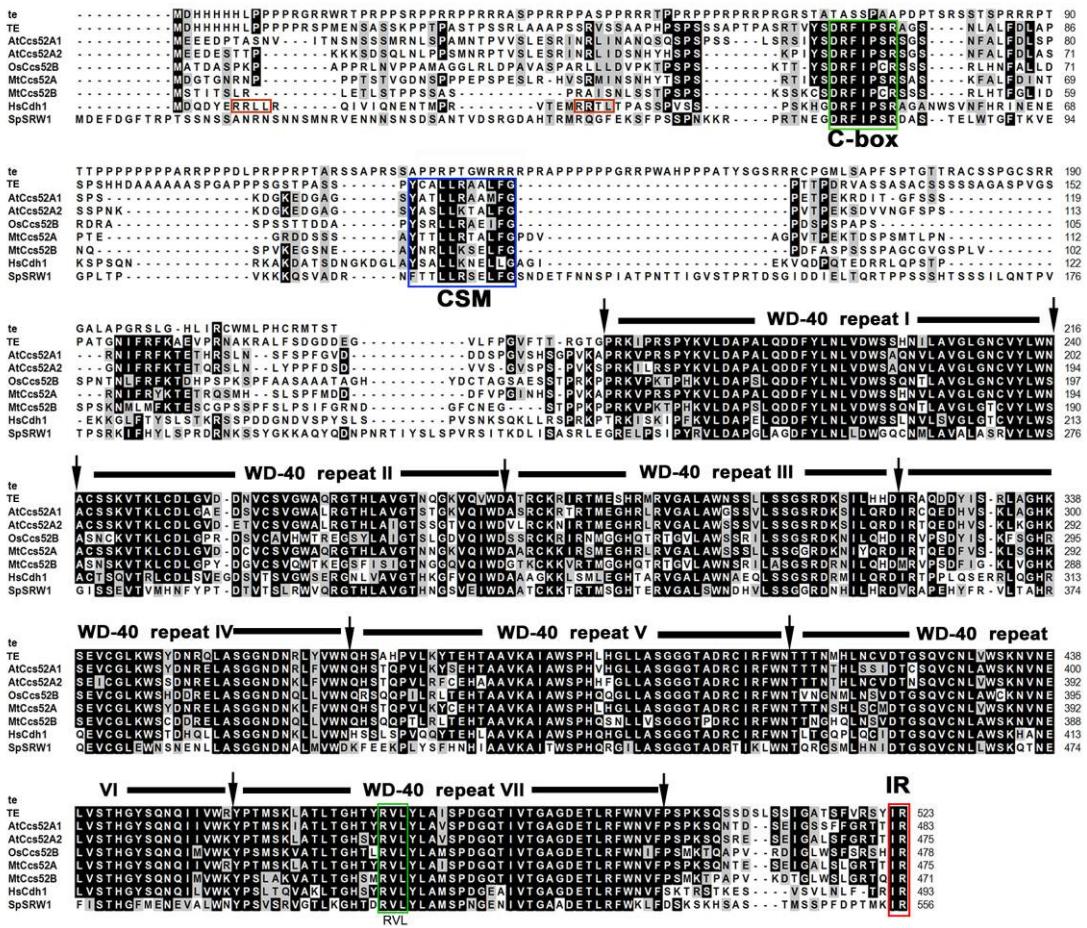
Qibing Lin^{1*}, Dan Wang^{1*}, Hui Dong^{2*}, Suhai Gu¹, Zhijun Cheng¹, Jie Gong², Ruizhen Qin¹, Ling Jiang², Gang Li³, Jiu Lin Wang¹, Fuqing Wu¹, Xiuping Guo¹, Xin Zhang¹, Cailin Lei¹, Haiyang Wang¹, Jianmin Wan^{1,2}

¹National Key Facility for Crop Gene Resources and Genetic Improvement, Institute of Crop Science, Chinese Academy of Agricultural Sciences, Beijing 100081, China

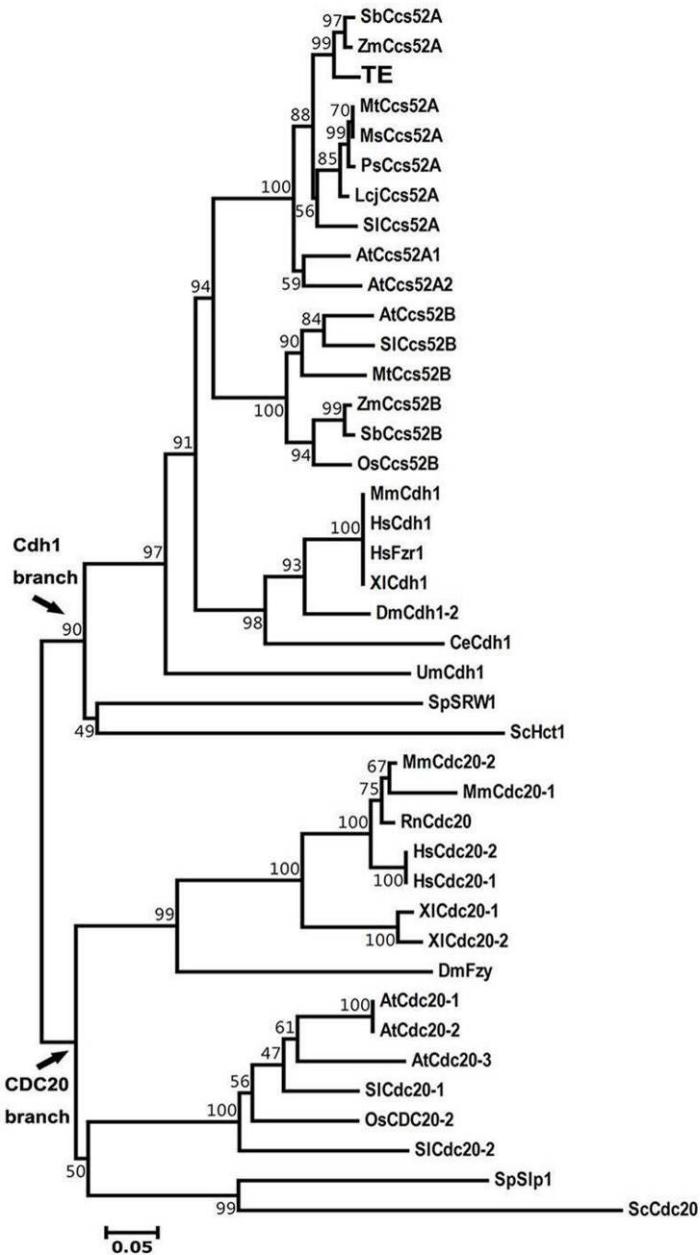
²National key Laboratory for Crop Genetics and Germplasm Enhancement, Jiangsu Plant Gene Engineering Research Center, Nanjing Agricultural University, Nanjing 210095, China

³Department of Molecular, Cellular, and Developmental Biology, Yale University, New Haven, Connecticut 06520-8104

Supplementary Figures

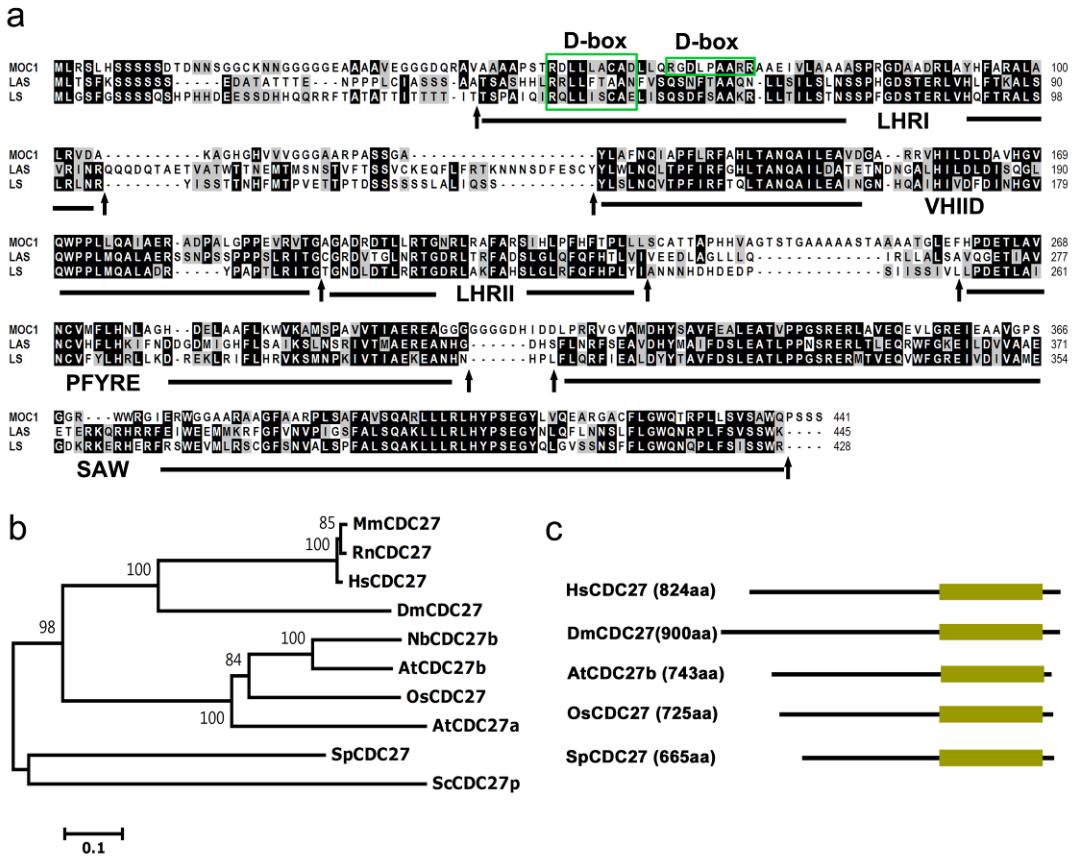


Supplementary Figure S1 | Protein sequence alignment of Cdh1-like proteins of *S. pombe* (SpSRW1), *H. sapiens* (HsCdh1), *A. thaliana* (AtCcs52A1, AtCcs52A2), *M. truncatula* (MtCcs52A, MtCcs52B), *O. sativa* (TE, OsCcs52B) and the truncated te protein. The conserved domains (seven WD-40 repeats) and motifs (C-box, CSM, IR, and RVL) are marked. In addition, the two degradation signals are also marked by two red boxes in the N-terminal region of HsCdh1.

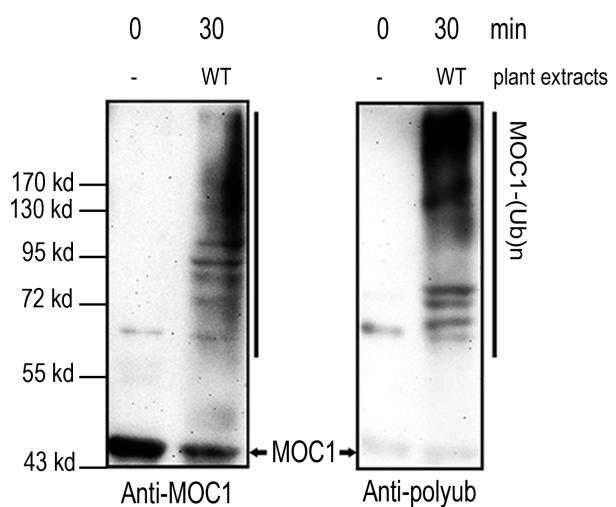


Supplementary Figure S2 | Phylogenetic analysis of TE protein. Protein sequences were aligned with CLUSTAL W in MEGA4.0⁴⁹ and the phylogenetic tree was analyzed by MEGA4.0⁴⁹. Accession numbers presented here are the following: SpSlp1, P78972; ScCdc20, P26309; SlCdc20-1, CBH19893; OsCDC20-2, BAF15668.1; SlCdc20-2, CBH19894; MmCdc20-1, XP 139652; HsCdc20-1, NP 001246; HsCdc20-2, A56021; MmCdc20-2, NP 075712; RnCdc20, NP 741990; XIcdc20-1, AAH42288; XIcdc20-2, AAC41376; DmFzy, NP 477501; AtCdc20-1, At4g33260; AtCdc20-2, At4g33270; AtCdc20-3, At5g27570; ScHct1, P53197; UmCdh1, AY118173; SpSRW1, O13286.1;

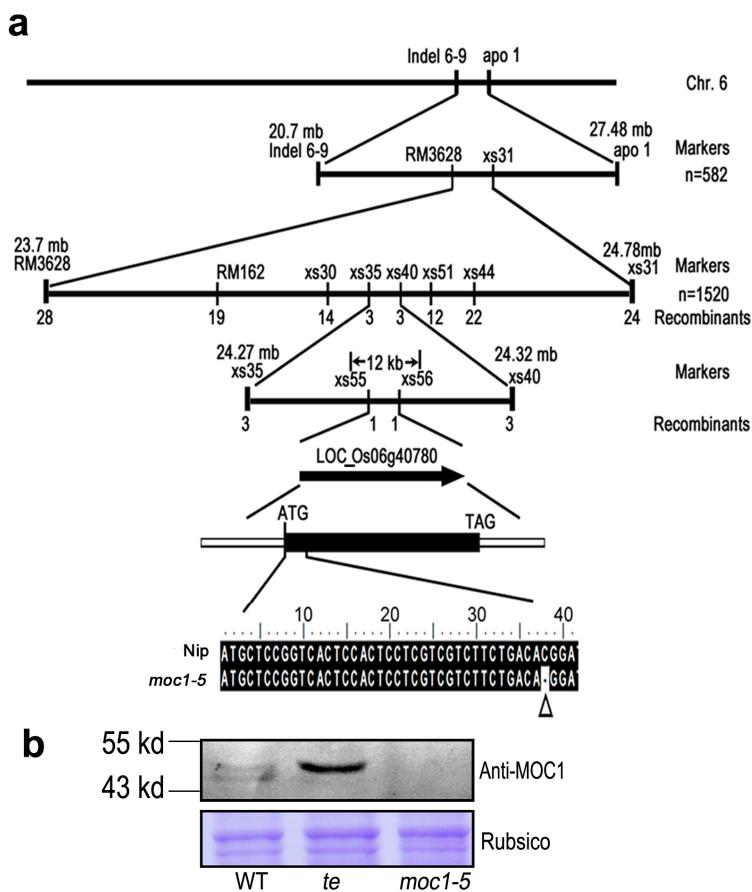
CeCdh1, NP 496075; XlCdh1, CAA74576; HsCdh1, NP 057347; HsFzr1, Q9UM11.2; MmCdh1, NP 062731; DmCdh1-2, NP 726941; OsCcs52B, LOC_Os01g74146; AtCcs52A2, At4g11920; AtCcs52A1, At4g22910; SlCcs52A, CBH19891; MtCcs52A, AF134835; TE, LOC_Os03g03150; ZmCcs52A, ACN36196; ZmCcs52B, ACG33710; AtCcs52B, At5g13840; MtCcs52B, AY357299; SpSrw1, O13286; SlCcs52B, CBH19892; LcjCcs52A, DQ059035.1; PsCcs52A, DQ059036; MsCcs52A, AF079404; SbCcs52A, XP_002468612.1; and SbCcs52B, XP_002457025.1. *Sp*, *S. pombe*; *Sc*, *S. cerevisiae*; *Sl*, *Solanum lycopersicum*; *Mm*, *Mus musculus*; *Hs*, *Homo sapiens*; *Rn*, *Rattus norvegicus*; *Xl*, *Xenopus laevis*; *Dm*, *D. melanogaster*; *At*, *A. thaliana*; *Um*, *Ustilago maydis*; *Ce*, *Caenorhabditis elegans*; *Os*, *Oryza sativa*; *Mt*, *M. truncatula*; *Zm*, *Zea mays*; *Lcj*, *Lotus corniculatus* var. *Japonicus*; *Ps*, *Pisum sativum*; *Ms*, *Medicago sativa*; *Sb*, *Sorghum bicolor*.



Supplementary Figure S3 | Phylogenetic and protein sequence analysis of MOC1 and OsCDC27. **(a)** Sequence alignment of MOC1, LAS and LS proteins. The conserved domains (LHRI, VHIID, LHRII, PFYRE and SAW) were marked. The typical degradation box (D-box) is marked by a green box in the N-terminal region of MOC1, LAS and LS. **(b)** Phylogenetic analysis showing that OsCDC27 is the rice homolog of HsCDC27, DmCDC27, AtCDC27a, AtCDC27b, and SpCDC27. Protein sequences were aligned with CLUSTAL W in MEGA4.0⁴⁹ and the phylogenetic tree was constructed by MEGA4.0⁴⁹. Accession numbers presented here are the following: HsCDC27, NP_001247.3; MmCDC27, NP_663411.2; RnCDC27, NP_001019964.1; DmCDC27, NP_648093.2; SpCDC27, NP_594604.2; ScCDC27p, NP_009469.1; AtCDC27b, NP_849994.1; AtCDC27a, NP_188253.3; OsCDC27, NP_001058099.1; NbCDC27b, BAF64845.1. **(c)** OsCDC27 has the same conserved TPR domain (indicated as green boxes) as HsCDC27, DmCDC27, AtCDC27b, and SpCDC27 in their C-terminal region.



Supplementary Figure S4 | Ubiquitination analysis of MOC1. *In vitro* ubiquitination assay showing that MOC1 is poly-ubiquitinated (represented by multiple up-shifted bands) by WT plant extracts and can be detected by antibodies against MOC1 or polyub antibodies on Western blots.



Supplementary Figure S5 | Mapping of the *moc1-5* allele. (a) Mapping of *moc1-5*. (b) Western blot analysis showing the absence of MOC1 proteins in *moc1-5* plants (top). Rubisco staining showing a roughly equal loading of proteins (bottom).

Supplementary Tables

Supplementary Table S1 | Molecular markers used for mapping of the *TE* gene

Marker	Type	Primer sequences (5' → 3')	
		Forward primer	Reverse primer
g41	SSR	CGCTCTGAAATCCGATATTGC	GGCTCTCTCCTCCTCGTTGC
RM22	SSR	GGTTTGGGAGCCCATAATCT	CTGGGCTTCTTCACTCGTC
RM523	SSR	AAGGCATTGCAGCTAGAAGC	AAGGCATTGCAGCTAGAAGC
g62	InDel	GCATAACATCAAAGCCATGCTA	GCTCCAATTGCGATCTCGTC
g105	InDel	GCCCTTCTCATTGCCTCG	CTCTTCTCGCTTCTCTTCG
g118	InDel	ATGTACAATAGTATTCCCACGTC	AAAACAAGGTTAACGGTAGTG

Supplementary Table S2 | Molecular markers developed in this study for mapping of the *moc1-5* allele

Marker	Type	Primer sequences (5' → 3')	
		Forward primer	Reverse primer
Indel 6-9	InDel	GTGCGAATTCCCTCACCTC	CGACATGGCTGGTCTCTCTC
xs31	InDel	TGCCAATTAGCTCGATTCTG	TGGTCAAAATTGTTCAAACCTCA
apo1	InDel	CCGGTTTGGTTGTCTCAG	ATTCCTTGCTGGGCTCCTTT
RM3628	SSR	GCCCTAGACACACCCGTACC	TGCCAGATCAGAAATCATGC
RM162	SSR	GTGGTAGGGCGAAATGATCTGC	ATCACGCGCTCCTACCTCACC
xs30	InDel	CAGCCGATTGGACTCGAT	AACACCTGCCGAACACC
xs35	InDel	TAAAAGAAAGGTTACTCATGGATT	TAATGCCTGCTGCATCTTAGG
xs40	InDel	TCCTCACACACAAGCACACA	TCAAATTGGCATGCATTGAT
xs51	InDel	AAGTCGCCGGAGAGAGAAG	CAGCGTCATGGTTCTGAAAGCCA
xs44	InDel	TTAATTAAATCACCAATGCAATCTATGA	CCAGCAACTTTGCTTGTG
xs55	InDel	CTTAGATTGACACAACCTGCAC	ATTCTGCAGTGCACGAGCTA
xs56	InDel	AACTATAGATCGGGATCGCTCA	TTCAAAGAAACGAAATCAACG

Supplementary Table S3 Primers used for construction of protein expression plasmids

Fragment or plasmid	Forward primer (5' → 3')	Reverse primer(5' → 3')
TE	CTAGGAAGATCTATGGATC ACCACCAACCACCAAC	CTAGATCTCGAGTCACCGGATGT AGCTCCTAACAAATG
MOC1	CGCGGATCCATGCTCCGG TCACTCCACTCC	CGCAAGCTTCTACGACGACGACG GCTGCCA
pMAL-c2x::MBP-MOC1-m	GCGGACTTGGCGCTGGCG TGCGCGGACCTG	CGTCGACGGCGCCGCCGCCGCA ACGGCAC

Supplementary Table S4 Primers used for construction of yeast two-hybrid assay plasmids

Fragment	Forward primer (5'→3')	Reverse primer (5'→3')
OsCDC27	AGTGAATTCATGGAAACCTAATGGTGGAC	ATAGGATCCTTAAATCTCATCATCATCATCC
MOC1	AGTGAATTCATGCTCCGGTCACTCCACTCCTC	AATGGATCCCTACGACGACGACGGCTGC
MOC1-m	AGTGAATTCATGCTCCGGTCACTCCACTCCTC	AATGGATCCCTACGACGACGACGGCTGC
MOC1d	AGTGAATTGGCCATGGCACGTGTC	AATGGATCCCTACGACGACGACGGCTGC
TE	GGAATTCCATATGATGGATACCACCAACCACCA CCTG	CTAGGAAGATCTCACCGGATGTAGCTC CTAACAAATG

Supplementary Table S5 Primers used in Real time Quantitative RT-PCR

Gene Name	Type	Primer sequences (5' → 3')	
		Forward primer	Reverse primer
Actin	qRT-PCR	TCGTCTGCGATAATGGAACTG	CCGACAATGCTGGGGAAG
TE	qRT-PCR	TGGTCTTCGCATAATATCCTTG	TGTCCCTACAGCAAGGTGAGT
OsTB1	qRT-PCR	CCTACCATGAGAGAAGAGACCA	GTA GTGGGCTATGATCAGATGTG
OSH1	qRT-PCR	CTCAACACGCTCTCCATCTC	GCTTGAGCTCCTGATCCAC

Supplementary Reference:

49. Tamura, K., Dudley, J., Nei, M. & Kumar, S. MEGA4: Molecular Evolutionary Genetics Analysis (MEGA) software version 4.0. *Mol. Biol. Evol.* **24**, 1596-1599 (2007).