

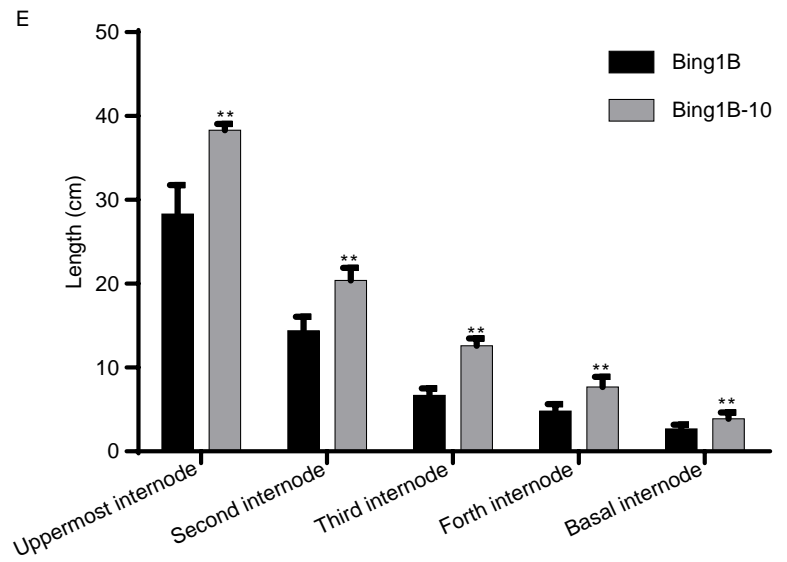
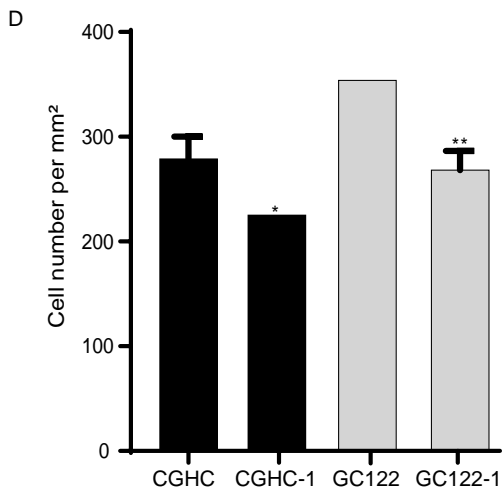
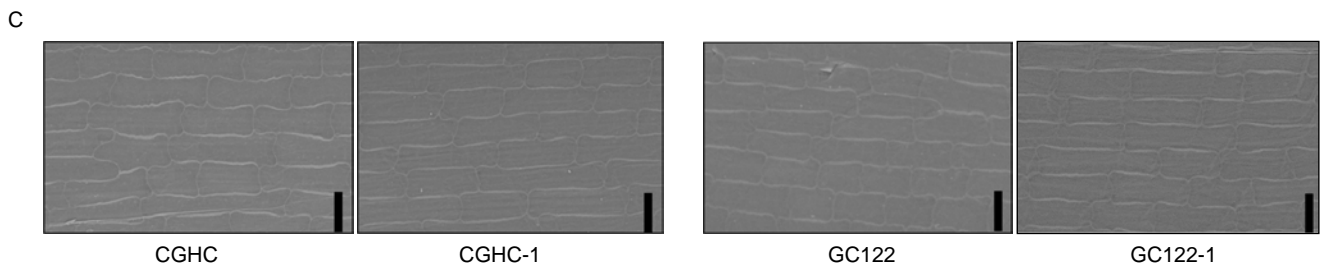
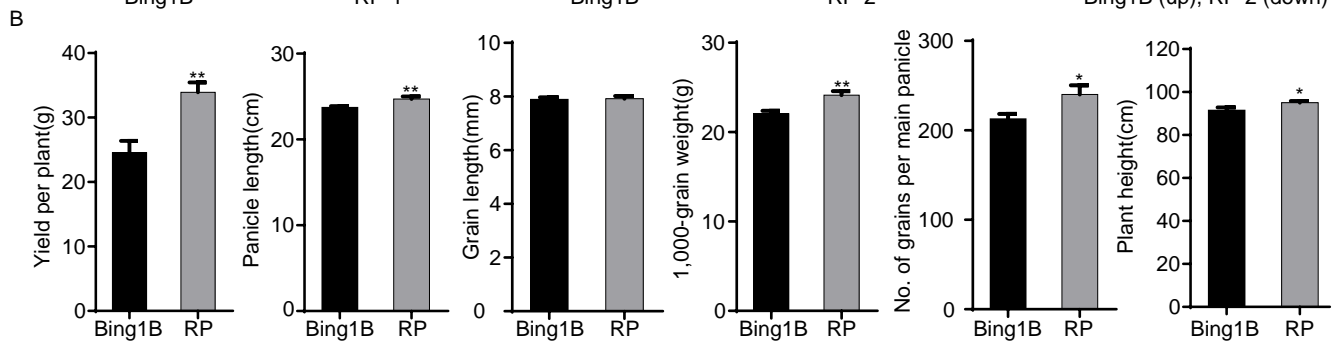
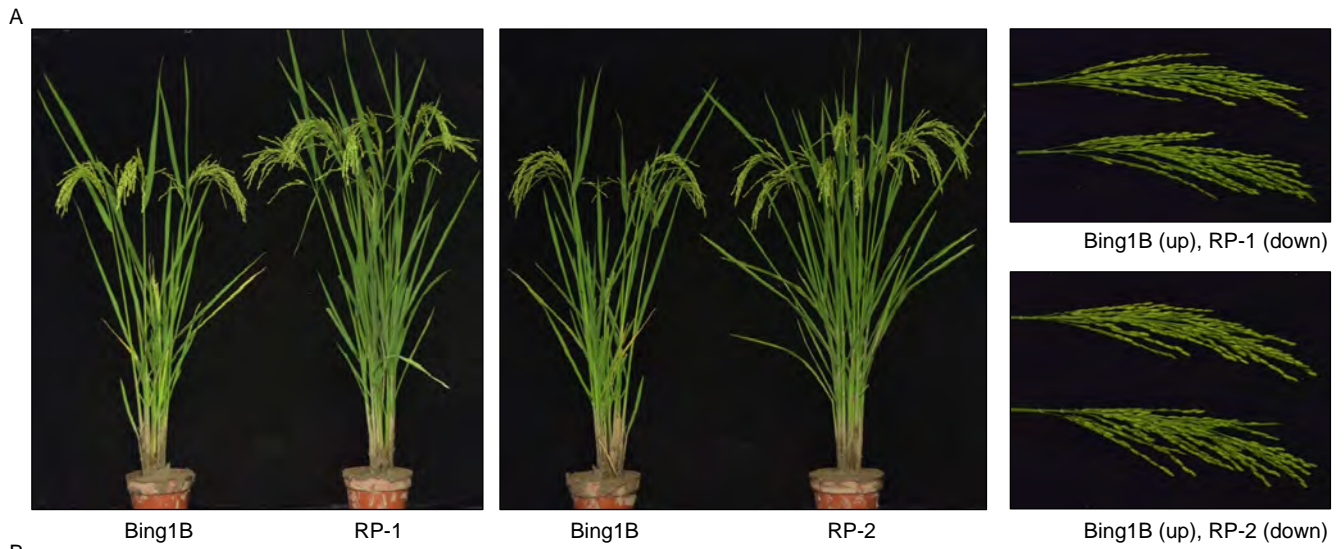


Supplementary Information for

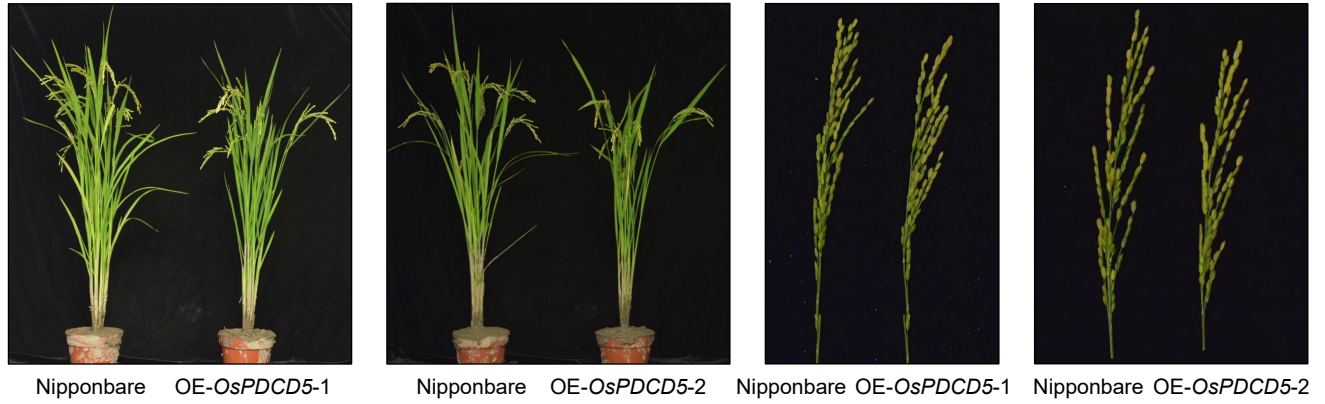
***OsPDCD5* negatively regulates plant architecture and grain yield in rice**

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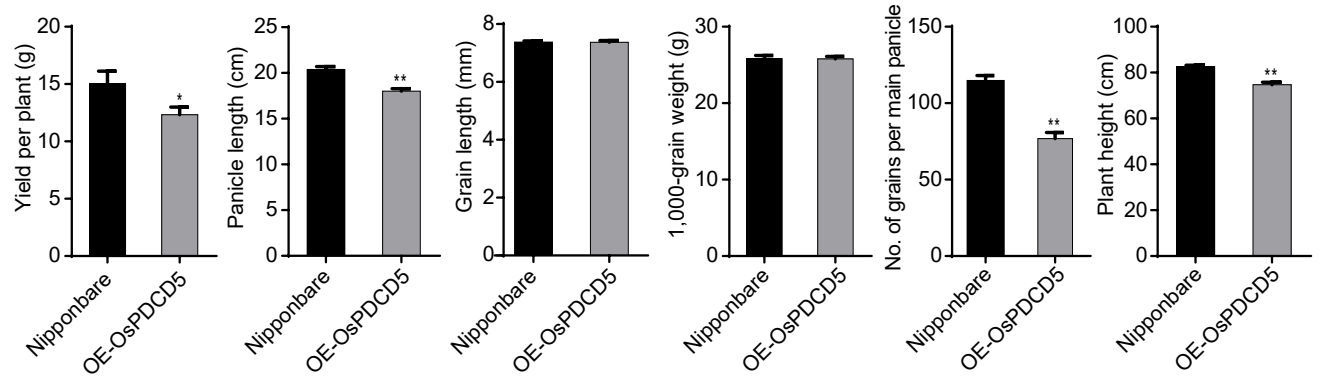
Xiaojin Luo<sup>1,3\*</sup>, Longping Yuan<sup>2\*</sup>, Jinshui Yang<sup>1\*</sup>, Bingran Zhao<sup>2\*</sup>  
Email: luoxj@fudan.edu.cn, lpyuan@hhrc.ac.cn, jsyang@fudan.edu.cn and  
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F



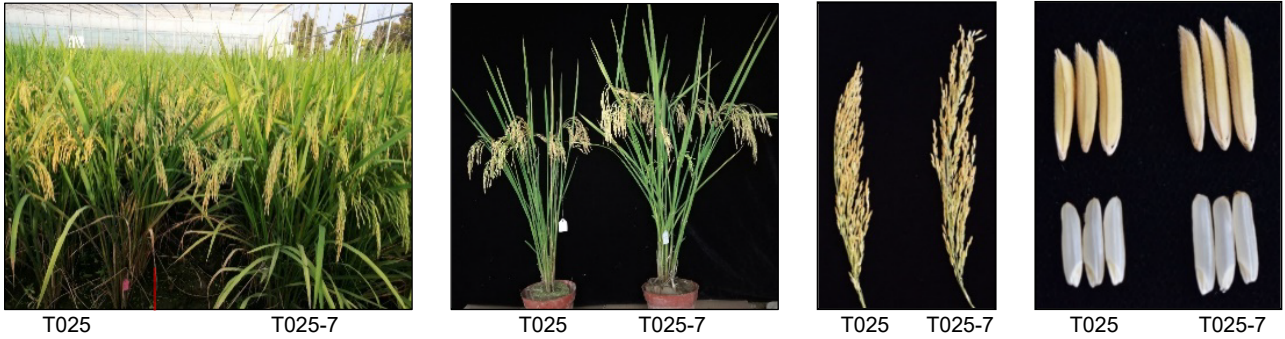
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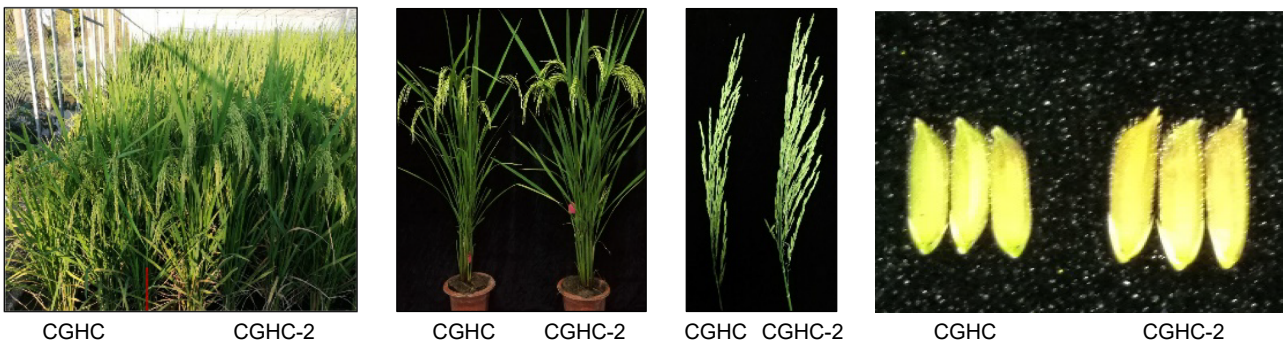
Target site on *OsPDCD5*

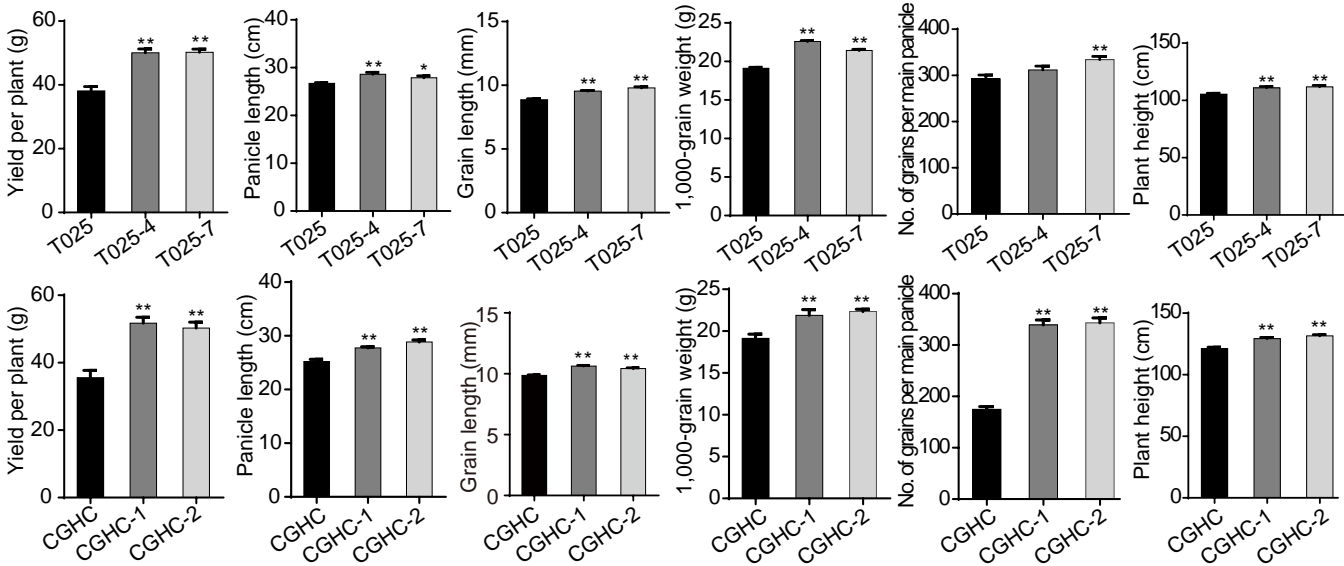
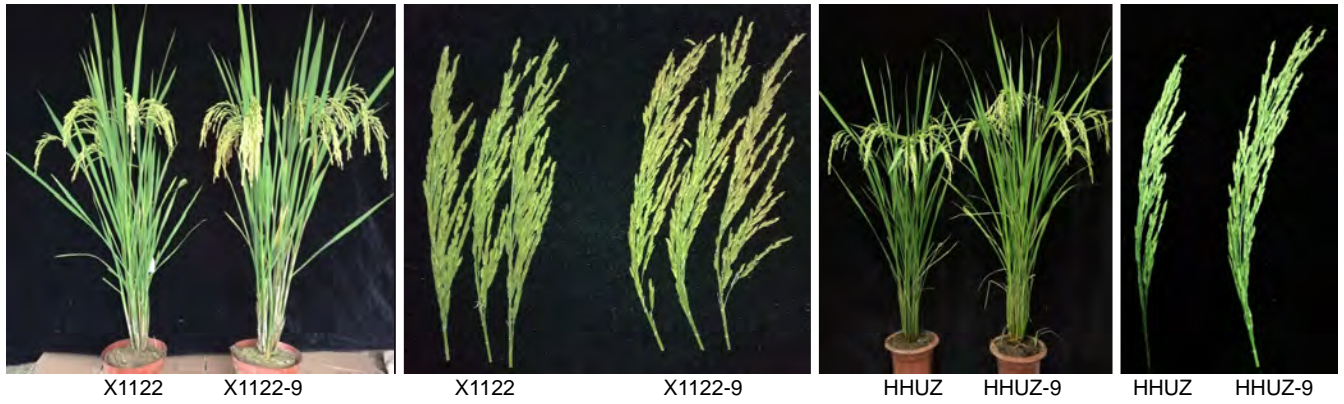
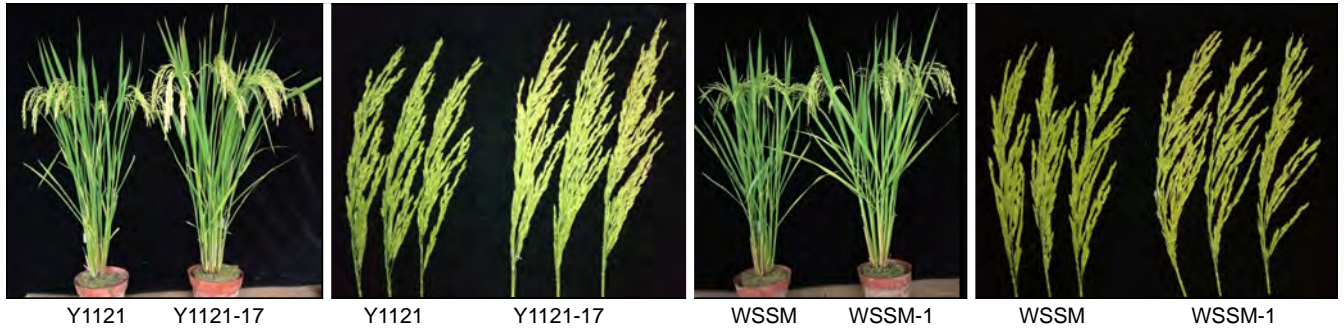
5' ...ACCCAGAGTTGGAAGCTATCAGGCAGAGG... 3' T025  
 5' ...ACCCAGAGTTGGAA - - - TCAGGCAGAGG... 3' T025-7

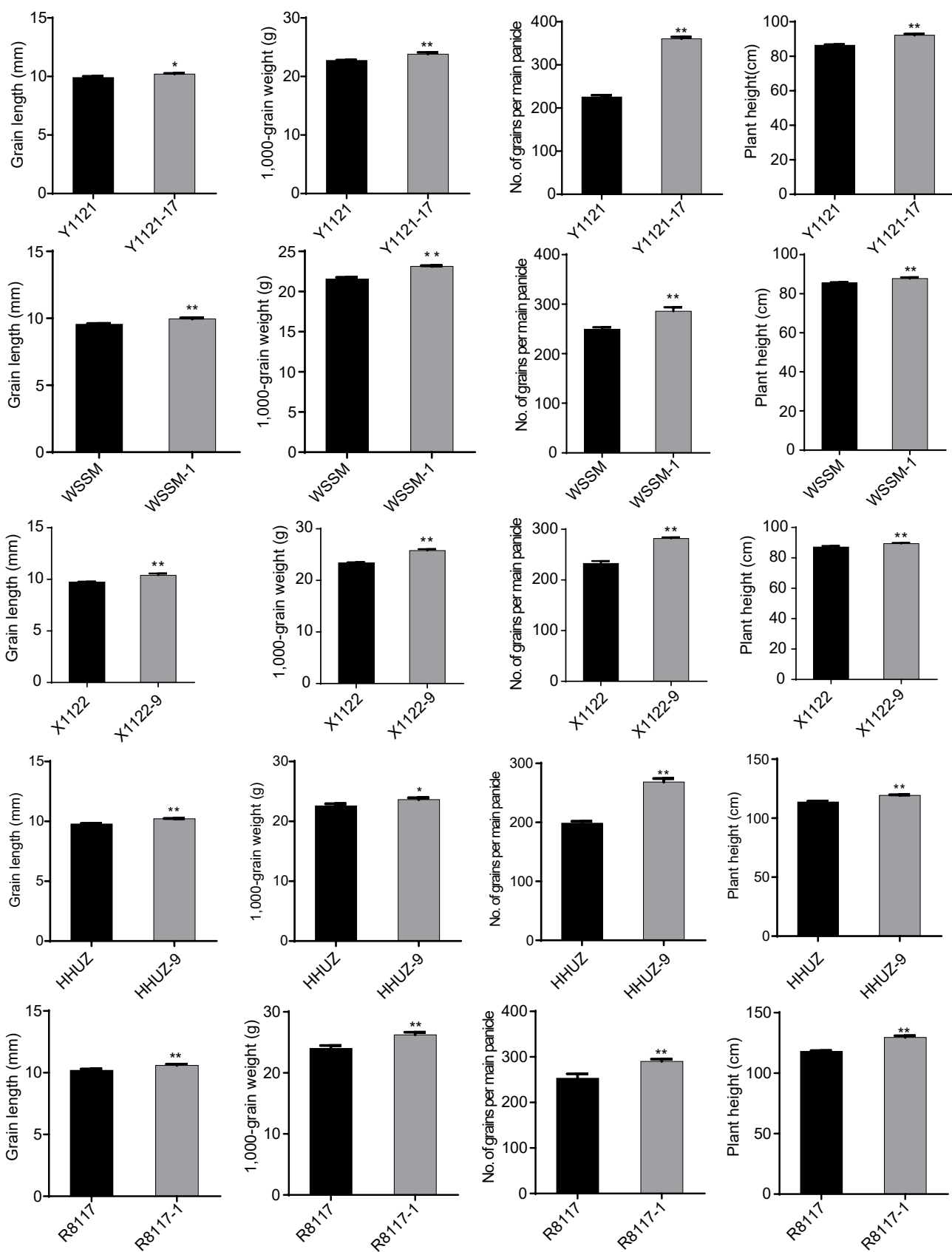


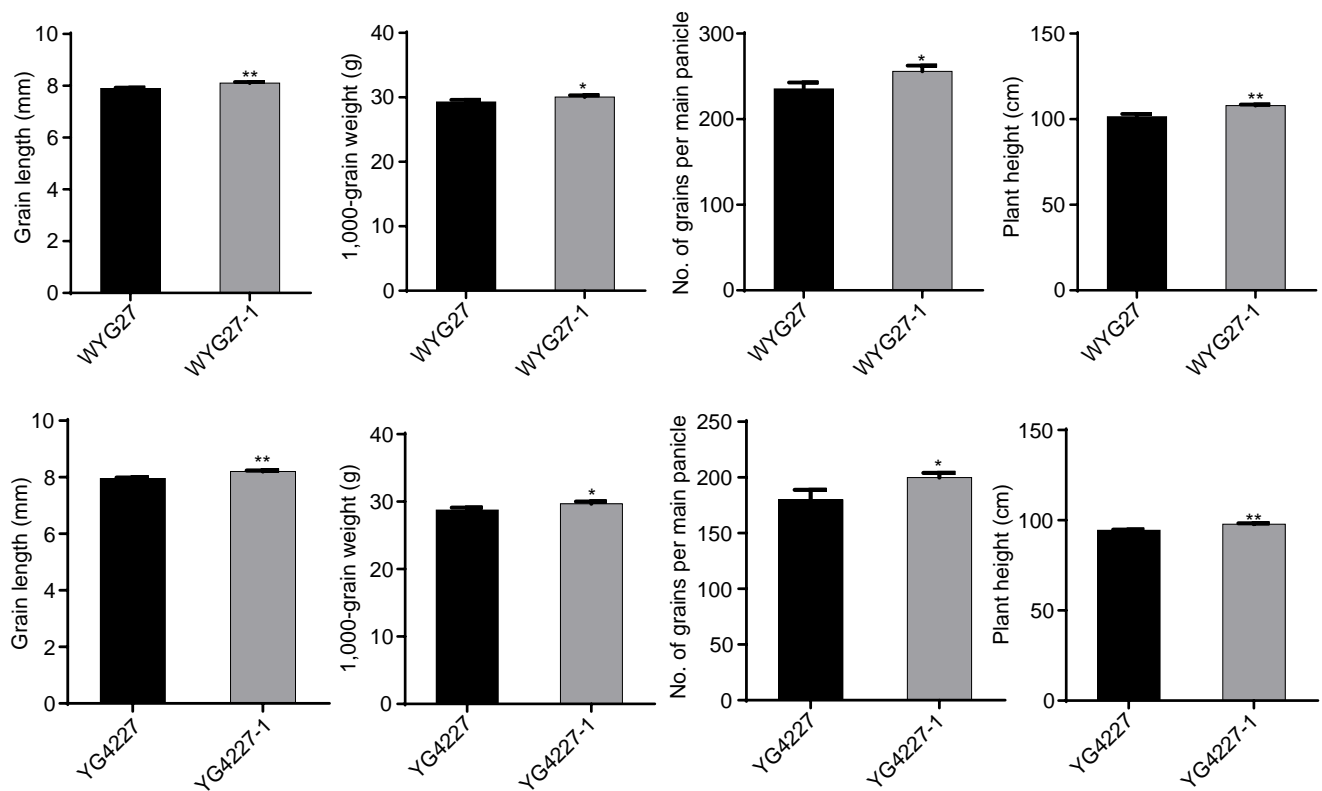
Target site on *OsPDCD5*

5' ...ACCCAGAGTTGGAAGCTATCAGGCAGAGG... 3' CGHC  
 5' ...ACCCAGAGTTGGAAGCTATCAGGC - GAGG... 3' CGHC-2

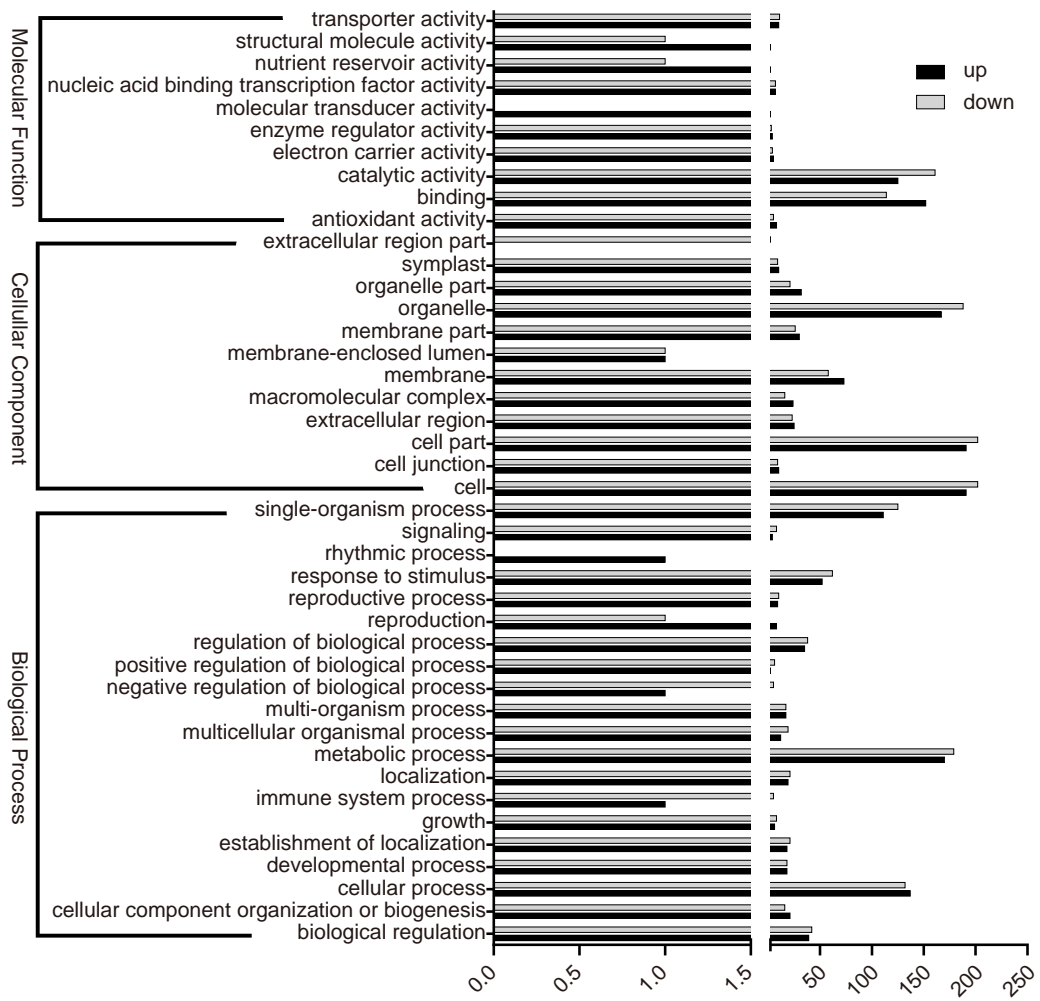




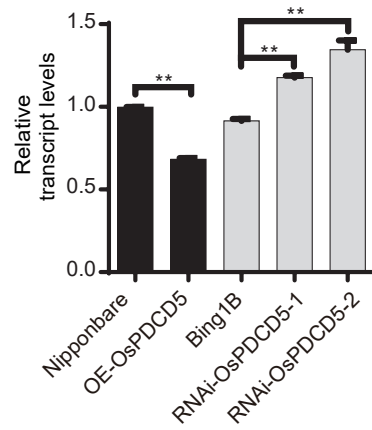




**Fig. S1.** Phenotypes of RNAi-*OsPDCD5*-RNAi lines (RP), *OsPDCD5* knockout lines and OE-*OsPDCD5* (transgenic line overexpressing *OsPDCD5*). (A) Plant architecture and panicles of Bing1B (wildtype) and RP-1 and RP-2 plants. RP-1 and RP-2 are individual plants of the same RNAi-*OsPDCD5* line. (B) Phenotypic traits of Bing1B and RP. (C) Scanning electron and light microscope photographs of the glume inner surfaces of mature seeds from wildtype and knockout lines CGHC, CGHC-1, GC122 and GC122-1. Scale bars are 50  $\mu$ m. (D) Cell density per square millimeter on the glume inner surfaces of CGHC, CGHC-1, GC122 and GC122-1. (E) Internode length of Bing1B and Bing1B-10. (F) Plant architecture and panicles of Nipponbare (wildtype) and OE-*OsPDCD5*-1 and OE-*OsPDCD5*-2 plants. (G) Phenotypic traits of Nipponbare and OE-*OsPDCD5*. OE-*OsPDCD5*-1 and OE-*OsPDCD5*-2 are individual plants of the same OE-*OsPDCD5* line. (H) Target site in the *OsPDCD5* sequence in *OsPDCD5* knockout line T025-7 and CGHC-2. Field state, plant architecture, panicles and grains of T025 (wildtype) and T025-7, CGHC (wildtype) and CGHC-2. Plant architecture and panicles of Y1121 (wildtype) and Y1121-17 (knockout), WSSM (wildtype) and WSSM-1 (knockout), X1122 (wildtype) and X1122-9 (knockout), HHUZ (wildtype) and HHUZ-9 (knockout), R8117 (wildtype) and R8117-1 (knockout), WYG27 (wildtype) and WYG27-1 (knockout), YG4227 (wildtype) and YG4227-1 (knockout). (I) Phenotypic traits of T025 and T025-7, CGHC and CGHC-2, Y1121 and Y1121-17, WSSM and WSSM-1, X1122 and X1122-9, HHUZ and HHUZ-9, R8117 and R8117-1, WYG27 and WYG27-1, YG4227 and YG4227-1. The data are the means  $\pm$  SEM (three replicates in D and E; over six replicates in B, G and I). \* $P < 0.05$ , \*\* $P < 0.01$  (Student's *t*-test).

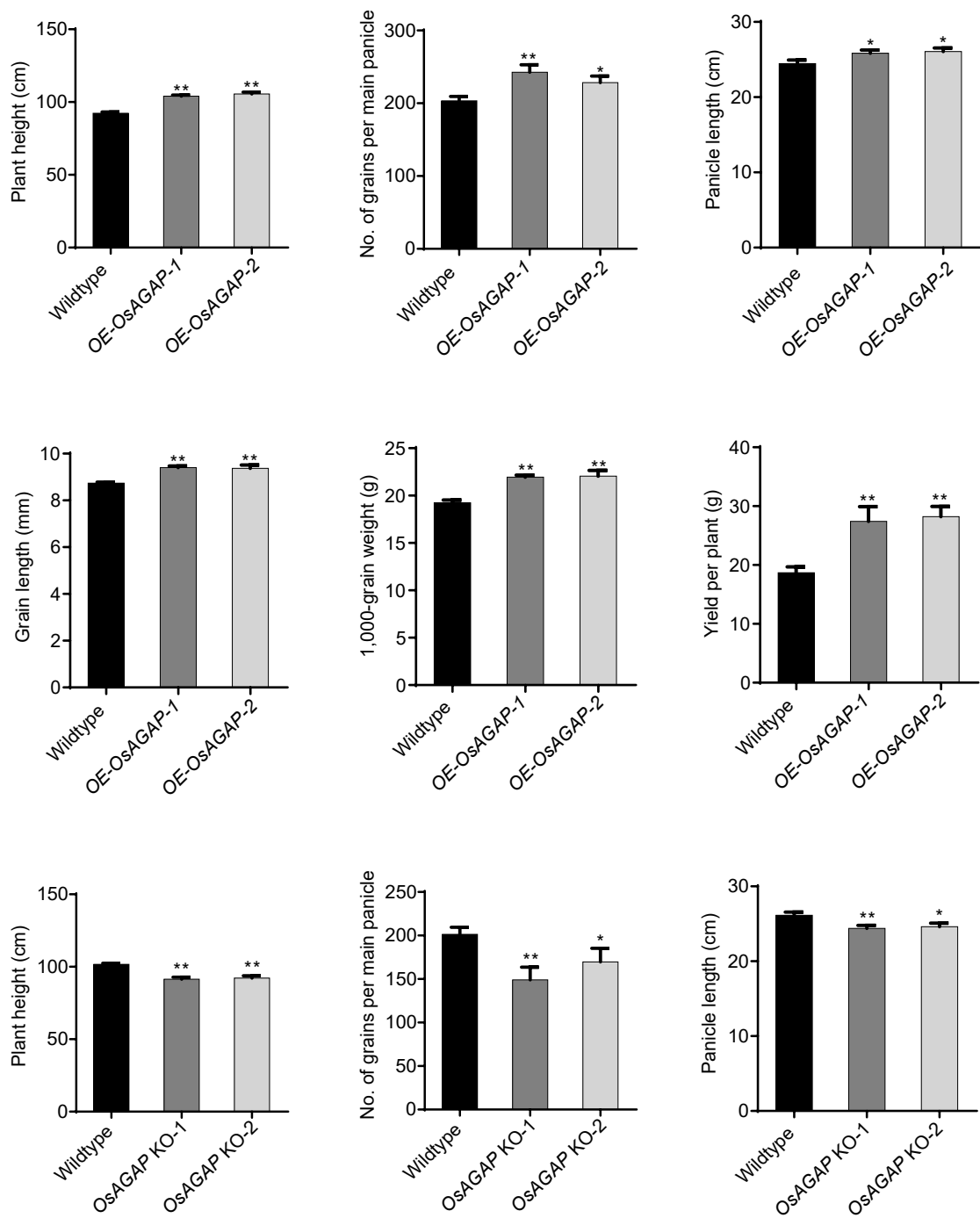


**Fig.S2.** GO functional enrichment analyzes of DEGs.



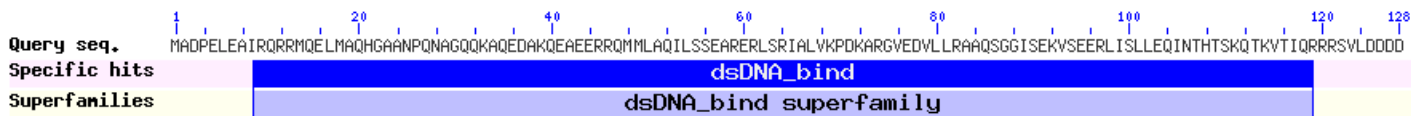
**Fig.S3.** Expression levels of *OsAGAP* in OE-*OsPDCD5* (transgenic line overexpressing *OsPDCD5*) and RNAi-*OsPDCD5* lines and the corresponding wildtypes Nipponbare and Bing1B by qPCR (three replicates). *Tubulin* was used as the loading control. The data are the means  $\pm$  SEM. \* $P < 0.05$ , \*\* $P < 0.01$  (Student's *t*-test).





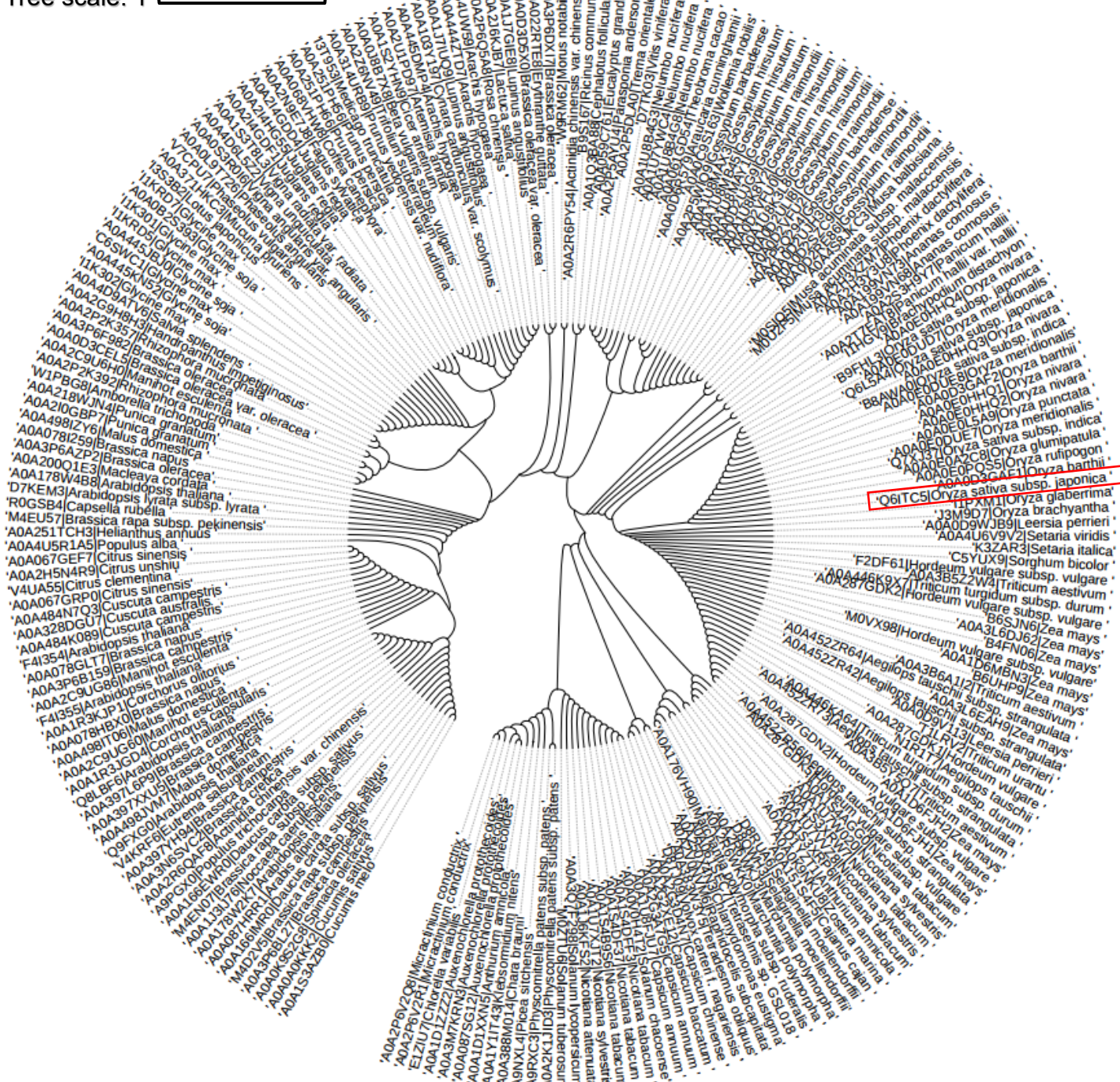
**Fig.S4.** Phenotypic traits of transgenic lines overexpressing *OsAGAP* (OE-*OsAGAP*-1 and OE-*OsAGAP*-2) and wildtype Bing1B (seven replicates). *OsAGAP* knockout lines (*OsAGAP* KO-1 and *OsAGAP* KO-2) and wildtype CGHC (over ten replicates). The data are the means  $\pm$  SEM. \* $P < 0.05$ , \*\* $P < 0.01$  (Student's *t*-test).

A

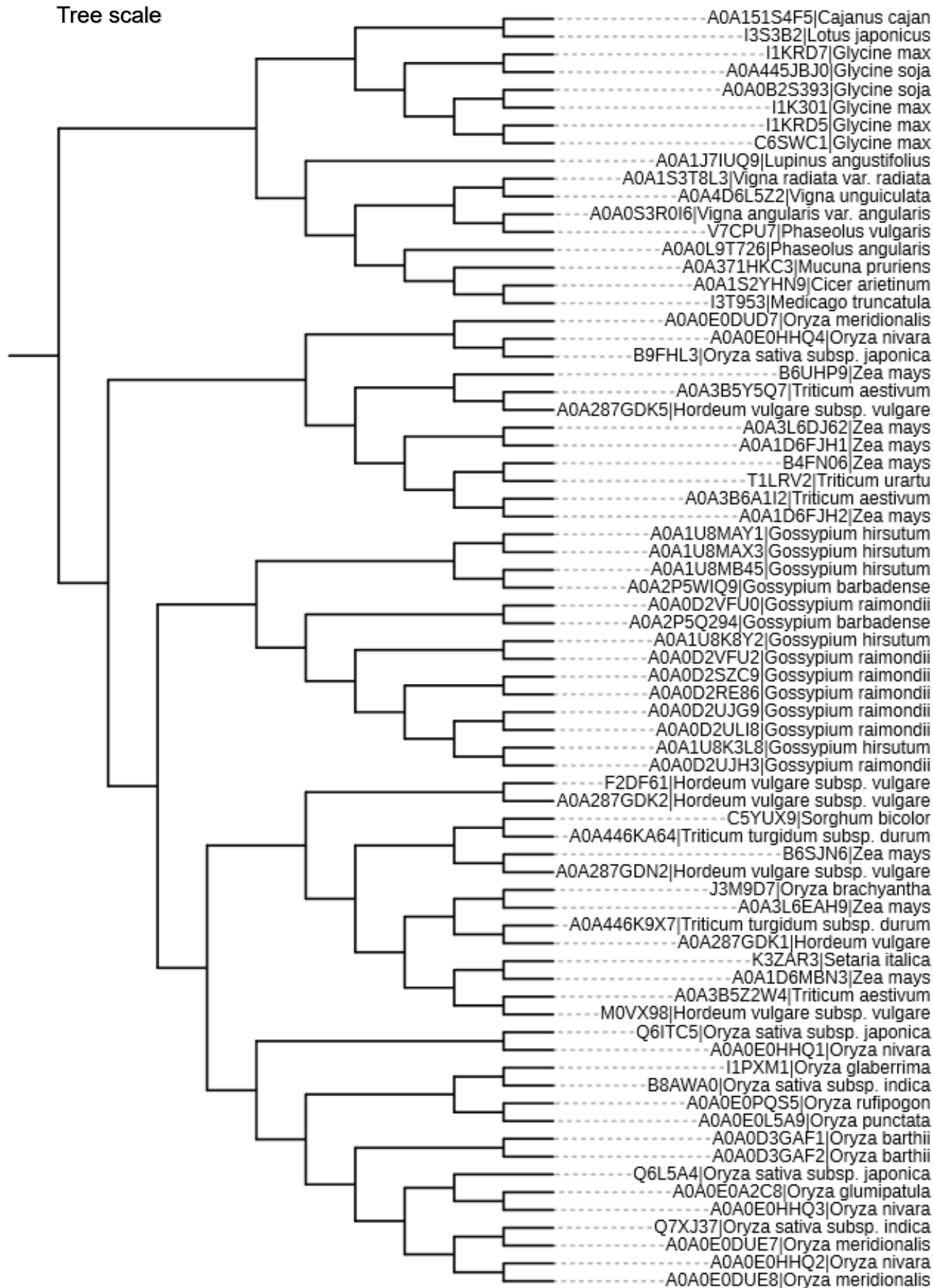


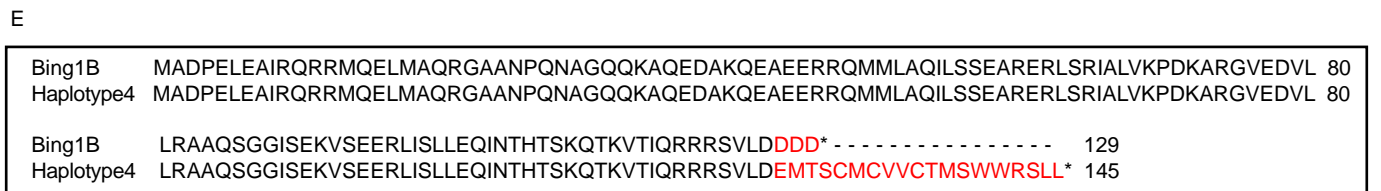
B

Tree scale: 1

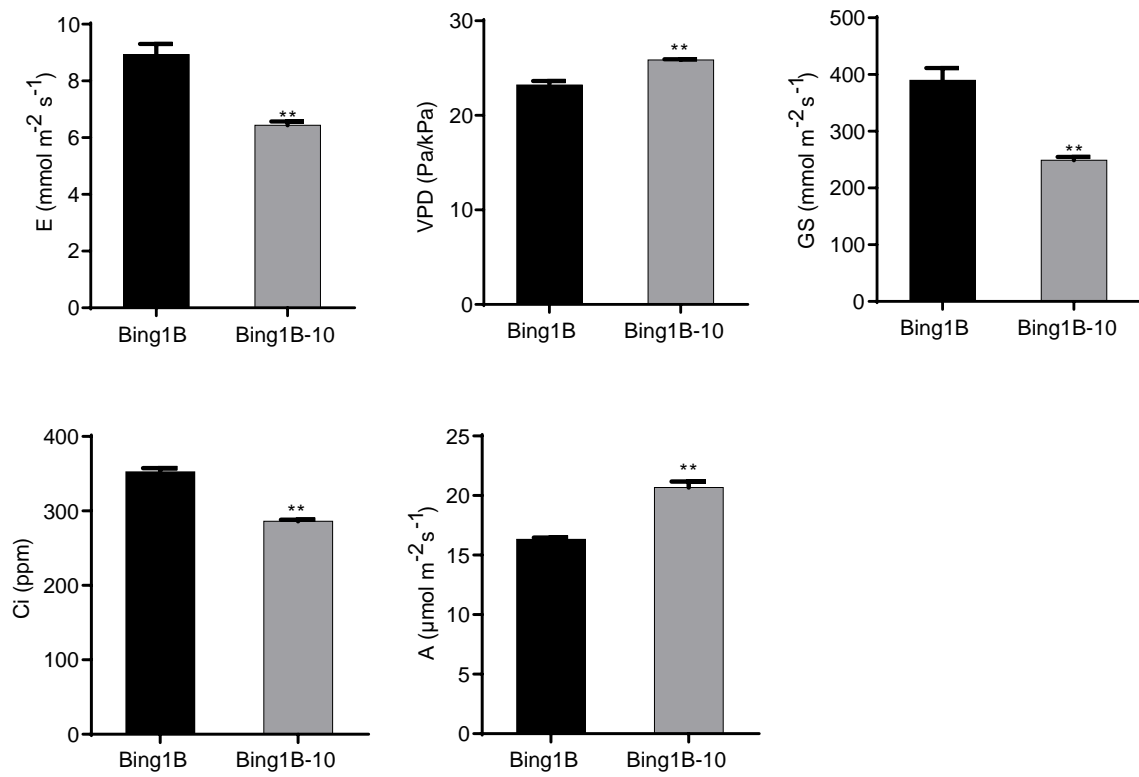


## Tree scale

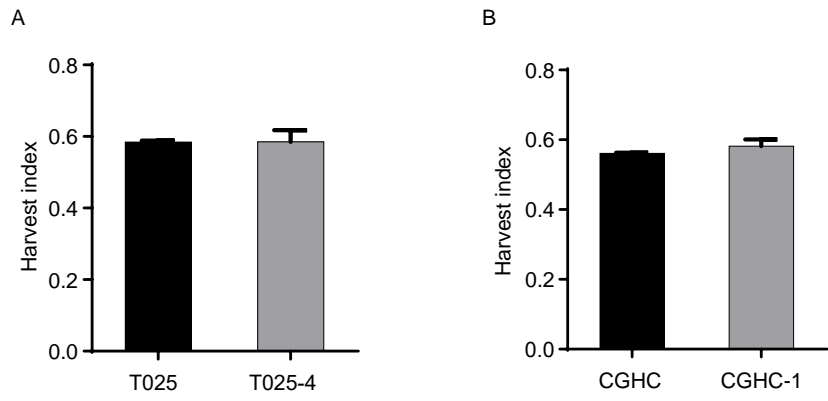




**Fig. S5.** *OsPDCD5* gene structure and conservation. (A) Predicted double-stranded DNA-binding domain of *OsPDCD5*. (B, C) *OsPDCD5* homologs are present in a large number of species (B) and in many common crops (C). *OsPDCD5* is marked by the red box. (D) Comparison of *OsPDCD5* haplotype3,4 and *OsPDCD5* in rice varieties edited in this study. (E) *OsPDCD5* protein alignment of Bing1B and Haplotype4 using the CLC sequence viewer7 multiple sequence alignment tool.



**Fig. S6.** Photosynthetic parameters of *OsPDCD5* knockout line Bing1B-10 and wildtype Bing1B at the tillering stage. E, transpiration rate; VPD, water pressure saturation deficit; GS, stomatal conductance; Ci, intercellular CO<sub>2</sub> concentration; A, net photosynthetic rate. The data are the means ± SEM (three replicates). \**P* < 0.05, \*\**P* < 0.01 (Student's *t*-test).



**Fig. S7.** Harvest index of *OsPDCD5* knockout lines. (A) Harvest index of T025 (wildtype) and T025-4 (knockout). (B) Harvest index of CGHC (wildtype) and CGHC-1 (knockout). (five replicates in A and B).

**Table. S1** Conservation of OsPDCD5 amino acid sequences among various plant species

Entry name	Organism	Identity	Score	E-value
I1PXM1	<i>Oryza glaberrima</i>	100.00%	611	8.00E-80
A0A0D3GAF1	<i>Oryza barthii</i>	100.00%	611	1.20E-79
B9FHL3	<i>Oryza sativa</i> subsp. <i>japonica</i>	98.50%	317	1.3E-35
Q7XJ37	<i>Oryza sativa</i> subsp. <i>indica</i>	98.40%	605	6.80E-79
K3ZAR3	<i>Setaria italica</i>	97.70%	601	2.80E-78
J3M9D7	<i>Oryza brachyantha</i>	96.90%	599	5.80E-78
C5YUX9	<i>Sorghum bicolor</i>	96.10%	590	1.30E-76
A0A287GDK5	<i>Hordeum vulgare</i> subsp. <i>vulgare</i>	95.50%	312	6.10E-35
A0A3B6A1I2	<i>Triticum aestivum</i>	93.70%	556	2.10E-71
B6SJN6	<i>Zea mays</i>	93.00%	564	1.10E-72
A0A0D2UJG9	<i>Gossypium raimondii</i>	86.70%	537	1.60E-68
A0A1S3T8L3	<i>Vigna radiata</i> var. <i>radiata</i>	82.80%	520	6.20E-66
A0A151S4F5	<i>Cajanus cajan</i>	79.50%	503	2.50E-63
.....	.....	.....	.....	.....
I3S3B2	<i>Lotus japonicus</i>	77.30%	489	3.40E-61

**Table. S2** Primers used to confirm knockout mutants

Name	Primers (5'-3')	Gene Name
OsU6-JC-S	CCTGGCTGTATTTGGTGGTT	<i>OsU6</i>
OsU6-JC-A	AGTTAGCTGGGCAATGTGGT	<i>OsU6</i>
Cas9-OsU6-F	GATGTGCAGTCAGGGACCAT	<i>Cas9</i>
Cas9-RNAi-R	GACTCGGTGCCACTTTTTCA	<i>Cas9</i>
PCD-Exon2,3,4-F	ACCATGTAAC TTCTCCTGATGGAAC	<i>OsPDCD5</i>
PCD-Exon2,3,4-R	GTTCAACAAAAATGACATCAAC	<i>OsPDCD5</i>
hyg (280)+	ACGGTGTCTCCATCACAGTTTGCC	<i>hygr</i>
hyg (280)-	TTCCGGAAGTGCTTGACATTGGGGA	<i>hygr</i>
Kana (270)+	CGGCTATGACTGGGCACAACAGACAAT	<i>Kana</i>
Kana (270)-	CTCGGCAGGAGCAAGGTGAGATGAC	<i>Kana</i>
35S-CX-S	ATCCTTCGCAAGACCCTTCCTC	



**Table. S3** List of rice cultivars used in this study

Type	Original producing area	Name on paper	Full name	Corresponding transgenic lines
<i>Xian/Indica</i>	China	Bing1B	Bing1B	Bing1B-10 (knockout line), RP-1, RP-2 (RNAi lines)
<i>Xian/Indica</i>	China	T025	Changhui T025	T025-1, T025-4, T025-7, T025-8, T025-10 (knockout lines)
<i>Xian/Indica</i>	China	CGHC	Changguihuicang	CGHC-1, CGHC-2 (knockout lines)
<i>Xian/Indica</i>	China	Y1121	Yeyue1121	Y1121-17 (knockout line)
<i>Xian/Indica</i>	China	WSSM	Wushansimiao	WSSM-1(knockout line)
<i>Xian/Indica</i>	China	X1122	Xin1121	X1122-9 (knockout line)
<i>Xian/Indica</i>	China	GC122	Guangchao122	GC122-1, GC122-2 (knockout lines)
<i>Xian/Indica</i>	China	HHUZ	Huanghuazhan	HHUZ-9 (knockout line)
<i>Xian/Indica</i>	China	R8117	R8117	R8117-1 (knockout line)
<i>Geng/japonica</i>	Japan	Nipponbare	Nipponbare	OE- <i>OsPDCD5-1</i> , OE- <i>OsPDCD5-2</i> (over-expression transgenic lines)
<i>Geng/japonica</i>	China	YG4227	YangGeng4227	YG4227-1(knockout line)
<i>Geng/japonica</i>	China	WYG27	WuYuGeng27	WYG27-1(knockout line)

**Table. S4** Primers used to construct vectors

Name	Primers (5'-3')	Gene Name
pPCD-5-Sall	GGgtcgacAGAAGAAGATGGAACAATAG	<i>OsPDCD5</i>
pPCD-3-BglII	ATAagatctACCATGGCTTCCCCGCGCGCTCA	<i>OsPDCD5</i>
PCD-GFP-NcoI	TATccatggATGGCTGACCCAGAGTTGG	<i>OsPDCD5</i>
PCD-GFP-SpeI	GGactagtGTCATCGTCGTCAAGGACGC	<i>OsPDCD5</i>
PCD-GFPC-EcoRI	TATgaattcATGGCTGACCCAGAGTTGG	<i>OsPDCD5</i>
PCD-GFPC-KpnI	GGggtaccGGTCATCGTCGTCAAGGACGC	<i>OsPDCD5</i>
PCD-RNAi5-BamHI	CGg gatccATGGCTGACCCAGAGTTGG	<i>OsPDCD5</i>
PCD-RNAi5-KpnI	GGggtaccCTAGTCATCGTCGTCAAGGAC	<i>OsPDCD5</i>
PCD-RNAi3-SpeI	GGactagtCTAGTCATCGTCGTCAAGGAC	<i>OsPDCD5</i>
PCD-RNAi3-SacI	GGgagctcATGGCTGACCCAGAGTTGG	<i>OsPDCD5</i>
PCD-GFPN-EcoRI	TATgaattcTATGGCTGACCCAGAGTTGG	<i>OsPDCD5</i>
PCD-GFPN-KpnI	GGggtaccCTAGTCATCGTCGTCAAGGA	<i>OsPDCD5</i>
M13R	CAGGAAACAGCTATGACC	
M13F	TGTAAAACGACGGCCAGT	
NGFP-Sequence	ATGGTCCGGACTCAGATCTCGA	<i>GFP</i>
GFPC-Sequence	CCATGGGCACTAGTATGGTC	<i>GFP</i>

**Table. S5** Primers used for the qPCRs

Name	Primers (5'-3')	Gene Name
Actin-F	TCTGGCATCACACCTTCTACA	<i>Actin</i>
Actin-R	GGAAGGCTGGAAGAGGAC	<i>Actin</i>
Tubulin-F	TACCGTGCCCTTACTGTTC	<i>Tubulin</i>
Tubulin-R	CGGTGGAATGTCACAGACAC	<i>Tubulin</i>
OsPDCD5-S2	CTGAGGAACGGCGGCAGATGAT	<i>OsPDCD5</i>
OsPDCD5-A2	GGTATTGATTTGCTCCAGAAGTG	<i>OsPDCD5</i>
OsAGAP-F	GAAGGAGCTATTGCACAG	<i>OsAGAP</i>
OsAGAP-R	CATATATAGCATTGGCGTG	<i>OsAGAP</i>
OsEXPA1-F	ACATGCCTCCTCTGGAAC	<i>OsEXPA1</i>
OsEXPA1-R	GTACATCACCGGCACGAT	<i>OsEXPA1</i>
OsEXPA5-F	CTTGATCACCCGCCAAAAT	<i>OsEXPA5</i>
OsEXPA5-R	TAAGCAGCACACAGGGCATA	<i>OsEXPA5</i>
OsEXPA10-F	GAACACCTTTGGAGCCAT	<i>OsEXPA10</i>
OsEXPA10-R	ATCTTCTCGAAGGACTGGAT	<i>OsEXPA10</i>
OsEXPA16-F	GTGAAGGTGAAAGGGTCGAG	<i>OsEXPA16</i>
OsEXPA16-R	CAAGAATGACACCCACATCG	<i>OsEXPA16</i>
06g47360-F	CACCTTCTACACCGTTTACAC	<i>LOC_Os06g47360</i>
06g47360-R	GTCGACGATCTTGACGGTGAC	<i>LOC_Os06g47360</i>
08g37930-F	CATCTGGAGTCGCCACCTTCTACAC	<i>LOC_Os08g37930</i>
08g37930-R	GTTGTCGACCATCTTCACGGTGAC	<i>LOC_Os08g37930</i>
06g03970-F	ATATTGCTCTGCGTTCTGCTG	<i>LOC_Os06g03970</i>
06g03970-R	ACAAGTGGCTGTGGTTGATTTG	<i>LOC_Os06g03970</i>
OsGA20ox1-S	TACGCCAGCAGCTTCACGG	<i>OsGA20ox1</i>
OsGA20ox1-A	TCCATCAGCTCCAGCGACA	<i>OsGA20ox1</i>
OsGA20ox3-S	CGCTCACCTTCTTCTCAACC	<i>OsGA20ox3</i>
OsGA20ox3-A	AGCCATTCTTTGCTTGATCCA	<i>OsGA20ox3</i>
OsGA2ox3-S	TCGTTGCAGGTTCTGACCA	<i>OsGA2ox3</i>
OsGA2ox3-A	CCTGTTGTCTCCAAGCCTT	<i>OsGA2ox3</i>
OsGA2ox4-S	CTCCTTCTCGTCATCGTC	<i>OsGA2ox4</i>
OsGA2ox4-A	GCAGCCTTCTTGTACTCCC	<i>OsGA2ox4</i>
OsGA2ox5-S	CCAGCACCTCATCGTCA	<i>OsGA2ox5</i>
OsGA2ox5-A	TGCTTCTGTACTCGCCGAA	<i>OsGA2ox5</i>
OsGA2ox6-S	GACTTCTCACCGTCTG	<i>OsGA2ox6</i>
OsGA2ox6-A	GCACCTTCTTCTGTACTCC	<i>OsGA2ox6</i>
OsGA3ox1-S	GATCTTCCATGTGCTCACC	<i>OsGA3ox1</i>
OsGA3ox1-A	GAATCATGCTCAACGCCGAT	<i>OsGA3ox1</i>

Name	Primers (5'-3')	Gene Name
OsGA3ox2-S	TCCAAGCTCATGTGGTCCGA	OsGA3ox2
OsGA3ox2-A	TCCTCTCCGCCTCGACTC	OsGA3ox2
EUI-F2	CTTCTCCTTCGATGTGATATCGC	OsEUI
EUI-R2	ACGCTGGTCTCCGACATGAG	OsEUI
OsGID1-F2	CAAGAGCCTCATCATCGTGT	OsGID1
OsGID1-R2	CATTGGACAACCTTGACGTG	OsGID1
OsGID2-F2	CTATCTCAGAGCTGGACACT	OsGID2
OsGID2-R2	CCTCCTGTTTCCGACAAATC	OsGID2
OsSPY-F	ATCGTCCTCTGGTCATTGGC	OsSPY
OsSPY-R	TCTTTACCAAGCTAGCAACC	OsSPY
SLR1-F1	GACGTCAACGAACGCTCAATT	OsSLR1
SLR1-R1	CGGAGTCCAGTCGTGATCT	OsSLR1
OsYUC3-F	CTGGTGACATCAAGGTACGG	OsYUC3
OsYUC3-R	ACTCCCGTCTTTAACCAG	OsYUC3
OsYUC9-F	CTGGCTCAAGAGTGATGACG	OsYUC9
OsYUC9-R	TCCTCGTAGCTGCCGTAGAT	OsYUC9
OsTAR2-F	CGCCCTACTACTCGTCATACC	OsTAR2
OsTAR2-R	GATTGTTCCGGGAGCAGAC	OsTAR2
OsIPT9-F	ACACCCGAACAACCATCGAA	OsIPT9
OsIPT9-R	GTCGACCCCACTTCTCTGTG	OsIPT9
OsIPT10-F	CTGCCTTCTCCTTGCCCTAC	OsIPT10
OsIPT10-R	CAGCATCTCTTCGCACCTCA	OsIPT10
LOG-F	CGGTTTGCTGATGCGTTCAT	OsLOG
LOG-R	TCGCTGACAGCCATGTCAAT	OsLOG
OsCKX4-F	GACCGACTACCTCCATCTCACA	OsCKX4
OsCKX4-R	GGTTGACATTGCTGACCTGC	OsCKX4
OsRR9/10-F	TCATGAGGACAGCCCAATTTCTA	OsRR9/10
OsRR9/10-R	TGCAGTAGTCTGTGATGATCAGGTT	OsRR9/10
OHK4-F	CAAGAACATATTGGTAGTTG	OsOHK4
OHK4-R	CCTTCCCTTCCATTGCTCGT	OsOHK4
OsAHP1-F	CAGAGATGGGTGCCTCAAGAC	OsAHP1
OsAHP1-R	CGCGCCAGTTTAATGTTTAGG	OsAHP1
OsAHP2-F	GCGCAACAAGTTTCAGACTATGC	OsAHP2
OsAHP2-R	GATCACATGCAGCTACACTCTTTTG	OsAHP2
OHK2-F	AGGGATGCTCCAAATGCTCA	OsOHK2
OHK2-R	AGATCAAAGGGCACCGTCTC	OsOHK2

Name	Primers (5'-3')	Gene Name
OHK3b-RT-F	CCTGCTTCTTGGTGTCTTAG	<i>OsOHK3b</i>
OHK3b-RT-R	AGCATCCTAGCTCTCATCA	<i>OsOHK3b</i>