

Supplementary Figure S1. Alignment of amino acid sequences of OsCDKAs and OsCDKB2.

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OsCDKA1 MEQYEKEEKIGEGTYGVVYRARDKVTNETIALKKIRLEQEDEGVNSTAIREISLLKEMHH 60
OsCDKA2 MEQYEKVEKIGEGTYGVVYKGRHRTNETIALKKIRLEQEDEGVNSTAIREISLLKEMQH 60
OsCDKB1 MEKYEKLEKVGEGTYGKVKYKAQDRATGQLVALKKTRLEMDEEGIPPTALREISILRLLSQ 60
OsCDKB2 MDLYEKLEKVGEGTYGKVKAREKATGRIVALKKTRLPEDEGVNSTALREVSLRRLMSQ 60

OsCDKA1 G-NIVRLHDVIHSE-----KRIYLVFEYLDLDLKKFMDSCEFA----KNPTLIKSYLYQ 110
OsCDKA2 R-NIVRLQDVVHKE-----KCIYLVFEYLDLDLKKHMDSSPDF-----KNHRIVKSFLYQ 109
OsCDKB1 SLYVVRLLSVEQAT-KNGKPVLYLVFEFLDTDLKKFVDAYRKGPNRPLPTNVIKSFLYQ 119
OsCDKB2 DSHVVRLLDLKQGQNKEGQTTILYLVFEYMDTDLKKFIRAHQRNLQKIPVPT--VKILMYQ 118

OsCDKA1 ILRGVAYCHSHRVLHRDLKPONLLIDRRTNALKLADFGGLARAFGIPVTRTFHEVVTLWYR 170
OsCDKA2 ILRGIAYCHSHRVLHRDLKPONLLIDRRTNSLKLADFGGLARAFGIPVTRTFHEVVTLWYR 169
OsCDKB1 LCKGVAHCHGHGVLHRDLKPONLLVDKEKGIKLIADLGLGRAFTVPMKSYTHEIVTLWYR 179
OsCDKB2 LCKGVAFCHGRGVLHRDLKPHNLLMDRKTMAKLIADLGLSRSTVPLKKYTHEILTLYR 178

OsCDKA1 APEILLGSRQYSTPVDMWSVGCIFAEMVNQKPLFPGDSEIDELFKIFRVLGTPNEQSWPG 230
OsCDKA2 APEILLGARHYSTPVDMWSVGCIFAEMVNQKPLFPGDSEIDELFKIFSIMGTPNEETWPG 229
OsCDKB1 APEVLLGSTHYSTGVDIWSVGCIFAEMVRRQALFPGDSELOQLLHIFRLLGTPTEEQWPG 239
OsCDKB2 APEVLLGAAHYSTPVDIWSVGCIFAELATNQPLFAGDSEVQQLLHIFKLLGTPNEQVWPG 238

OsCDKA1 VSSLPDYKSAFPKWQAQDLATI VPTLDPAGLDLLSKMLRYEPNKRITARQALEHEYFKDL 290
OsCDKA2 VASLPDYISTFPKWPSVDLATVPTLDSSGLDLSKMLRLDPSKRINARAALHEYFKDL 289
OsCDKB1 VTDLRDWH-EFPQWKQIILERQVPSLEPEGVLDLSKMLQYNPANRISAKAAMEHPYFDSL 298
OsCDKB2 VSKLPNWH-EYPQWNPSKVSVDLVHGLDADALDLEKMLQYEPKRI SAKKAMEHPYFNDV 297

OsCDKA1 EMVQ- 294
OsCDKA2 EVA-- 292
OsCDKB1 DKSQF 303
OsCDKB2 NKELY 302

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Amino acids sequence coded by 20 bp target sequence in sgRNA is underlined.

Supplementary Figure S2 Alignment of cDNAs of OsCDKAs and OsCDKB2.

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OsCDKA1      ATGGAGCAGTACGAGAAGGAGGAGAAGATTGGGGAGGGCACGTACGGGGTGGTGTACAGG 60
OsCDKA2      ATGGAGCAGTACGAGAAGGTGGAGAAGATCGGGGAGGGGACGTACGGGGTGGTGTACAAG 60
OsCDKB1      ATGGAGAAGTACGAGAAGCTGGAGAAGGTGGGGGAAGGGACGTACGGGAAGGTGTACAAG 60
OsCDKB2      ATGGACCTGTACGAGAAGCTGGAGAAGGTCGGGGAGGGGACGTACGGGAAGGTGTACAAG 60
              *****
OsCDKA1      GCGCGGG-ACAAGGTCACCAACGAGACGATCGCGCTCAAGAAGATCCGGCTTGAGCAGGA 119
OsCDKA2      G-GCAAGCACCGGCATACCAACGAGACGATCGCGCTCAAGAAGATCCGCCTGGAGCAGGA 119
OsCDKB1      GCGCAGG-ACAGGGCGACGGGGCAGCTGGTGGCGCTGAAGAAGACGAGGCTGGAGATGGA 119
OsCDKB2      GCGCGGG-AGAAGGCGACGGGGCGGATCGTGGCGCTGAAGAAGACGCGCTGCCGGAGGA 119
              * * * * *
OsCDKA1      GGATGAGGGCGTCCCTCCACCGCAATCCGCGAGATCTCGCTCCTCAAGGAGATGCATCA 179
OsCDKA2      GGACGAGGGCGTCCCTCCACCGCATCCGCGAGATCTCGCTGCTCAAGGAGATGCAGCA 179
OsCDKB1      CGAGGAGGGGATCCCGCCACCGCGCTGCGCGAGATCTCCATCCTCAGGCTGCTCTCCA 179
OsCDKB2      CGACGAGGGCGTGCCGCCGACGGCGCTCAGGGAGGTGTCGCTGCTCGGATGCTGTCCGA 179
              ** * * * * *
OsCDKA1      CG-GC---AACATCGTCAGGTTACACGATGTTATCCAC-----AGTGAGAAGCGCA--- 226
OsCDKA2      TC-GC---AACATCGTCAGGCTGCAGGACGTCGTGCAC-----AAGGAGAAATGCA--- 226
OsCDKB1      GTCGCTC-TACGTGCTCCGCCTCCTCTCCGTGAGCAGGCC---ACCAAGAACGGCAAGC 235
OsCDKB2      GG-ACTGCGACGTGGTGCCTGCTGACCTCAAGCAGGGGCAGAACAGGAGGGCCAGA 238
              * * * * *
OsCDKA1      -----TATATCTTGCTTTTGTAGTATCTGGATCTGGACCTAAAGAAGTTCATGGACTCTT 280
OsCDKA2      -----TATACCTCGTCTTCGAGTACCTCGACCTTGACCTCAAGAAGCACATGGACTCAT 280
OsCDKB1      CCGTCCTCTACCTCGTCTTCGAGTTCCTCGACACCGACCTCAAGAAGTTCGTGACGCTC 295
OsCDKB2      CCATCTCTACCTCGTCTTCGAGTACATGGACACCGACCTCAAGAAGTTCATCCGCGCCC 298
              * * * * *
OsCDKA1      GTC--CAGAGT-TTGCGAAAAACCCCACTTTAA-----TTAAGTCATATCTCTATC 328
OsCDKA2      CCC--CGGATT-TCAAGAACCACCGCA---TAG-----TCAAATCGTTCCTCTACC 325
OsCDKB1      ACCGCAAGGGCCCCAACCCCTCGCCCTCCCCACCAACGTCATCAAGAGCTTCTTGTATC 355
OsCDKB2      ACCGCCAGAATCTCCAGAAGATCCCGTGCACCG-----TCAAGATCCTGATGTACC 352
              * * * * *
OsCDKA1      AGATACTCCGCGGCGTTGCTTACTGTCACTTCTCATAGAGTTCTTCATCGAGATTTGAAAC 388
OsCDKA2      AGATTCTCCGGGGCATTGCGTACTGCCACTCGCACCGTGTCTCCACCGAGATTTGAAGC 385
OsCDKB1      AGTTATGCAAAGGAGTCCGACATGGCCATGGCCATGGTGTCTTCCACCGTATTTAAAGC 415
OsCDKB2      AGCTCTGCAAGGGCGTTGCTTTCTGACATGGCCGCGGTGTGCTGCACCTGACCTCAAGC 412
              ** * * * * *
OsCDKA1      CTCAGAATTTATTGATAGATCGGCGTACTAATGCACTGAAGCTTGCGAGACTTTGGTTTAG 448
OsCDKA2      CCCAGAACCTGCTGATAGATCGGCGTACCAACTCATTGAAGCTCGCGGACTTTGGGTGGA 445
OsCDKB1      CACAAAACCTGTTGGTCGACAAGGAAAAGGGGATATTGAAAATGCTGATCTTGGGTAG 475
OsCDKB2      CGCATAACCTGCTCATGGACCGCAAGACCATGGCGCTCAAGATCGCGGACCTCGGGCTCA 472
              * * * * *
OsCDKA1      CCAGGGCATTGGAATTCCTGTCCGCACGTTTACTCACGAGGTTGTAACCTTGTGGTATA 508
OsCDKA2      CCAGGGCATTGGCATTCTGTCCGGACATTTACTCACGAGGTTGGTACATTGTGGTATA 505
OsCDKB1      GTAGGGCGTTTCACTGTTCTTATGAAAAGCTACACACATGAGATTGTGACTCTTTGGTACA 535
OsCDKB2      GCCGCTCGTTCACTGTCCCTCTCAAGAAGTACACCCACGAGATCCTGACGCTGTGGTACA 532
              * * * * *
OsCDKA1      GAGCTCCAGAGATCCTTCTTGGATCAAGGACGATTTCTACACCAGTTGATATGTGGTCAG 568
OsCDKA2      GAGCACCTGAAATCTTCTTGGTGCAAGGCATTTATCCACCCCTGTTGACATGTGGTCAG 565
OsCDKB1      GAGTCTCTGAAGTTTGTCTTGGATCAACACATTACTCAACCGGTGTGACATTTGGTT-- 593
OsCDKB2      GGGCTCCCGAGGTTCTTCTTGGCGGGCACACTACTCCACTCCGGTTGACATCTGGTCTG 592
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OsCDKA1      TTGGTTGTATCTTTGCAGAAATGGTGAACCAGAAACCACTGTTCCCTGGTGATTCTGAGA 628
OsCDKA2      TTGGTTGCATTTTTGCTGAAATGGTGAATCAGAAGCCACTATTTCCCTGGAGATTCTGAGA 625
OsCDKB1      -----GTTGGGAAGTCC----- 605
OsCDKB2      TGGGCTGCATTTTTGCTGAGTTGGCCACTAACCAACCTCTTTTCGCTGGAGATTCCGAGG 652
                                     *** * **

OsCDKA1      TTGATGAATTATTTAAGATATTCAGGGTACTAGGAACTCCAAATGAACAAAGTTGGCCAG 688
OsCDKA2      TTGATGAACTCTTTAAGATTTTCAGTATTATGGGCACTCCAAATGAAGAACTTGGCCAG 685
OsCDKB1      -----TACTGAG-----GAGCAG---TGCCCTG 625
OsCDKB2      TTCAGCAGCTTCTGCACATTTTCAAGTTGCTGGGCACCCCAAATGAGCAAGTTTGGCCAG 712
                                     * * * * * ** * ** *

OsCDKA1      GAGTTAGCTCATTACCTGACTACAAGTCTGCTTTCCCAAGTGGCAAGCACAGGATCTTG 748
OsCDKA2      GTGTTGCTTCACTACCTGACTACATATCAACTTTCCCAAGTGGCCATCTGTGGATCTTG 745
OsCDKB1      GAGTAACTGATTTGAGGGACTG---GCATGAGTTTCCACAGTGG-AAGCCACAGATTTTA 681
OsCDKB2      GAGTGAGCAAGCTACCTAACTG---GCACGAGTACCCCAAGTGG-AATCC-----CTCGA 763
      * * * * * * * * * * * * * * * * * *

OsCDKA1      CAAC-TATTGTCCCTACTCTTG-----ACCCTGCTGGTTTGGACCTTCTCTCTAAAATGC 802
OsCDKA2      CAAC-CGTGGTCCCAACACTTG-----ATTCTTCAGGACTCGATCTTCTCTCTAAAATGC 799
OsCDKB1      GAACGTCAAGTCCCATCATTGG-----AGCCTGAAGGAGTTGACCTTTTATCGAAAATGC 736
OsCDKB2      AAGTGTCTGATCTTGTCCATGGTCTCGACGCTGATGCTCTTGATCTTCTTGAGAAAATGC 823
      * * * * * * * * * * * * * * * * * *

OsCDKA1      TTCGGTACGAGCCAAACAAAAGGATCACAGCTAGACAGGCTCTTGAGCATGAATACTTCA 862
OsCDKA2      TCCGTTTAGATCCAAGCAAAAGAATCAATGCCCGTCTGCCCTCGAGCACGAGTACTTCA 859
OsCDKB1      TCCAGTATAACCCAGCAAATCGGATCTCAGCAAAGGCTGCTATGGAACACCCCTACTTCA 796
OsCDKB2      TGCAGTACGAGCCGTCGAAGCGGATCTCTGCGAAGAAGCCATGGAGCATCCCTACTTCA 883
      * * * * * * * * * * * * * * * * * *

OsCDKA1      AGGACCTTGAGATGG-----TACAATGA 885
OsCDKA2      AGGACCTGGAAGTGG-----CGTAG--- 879
OsCDKB1      ACAGCCTCGACAAGTCCCAGTTCTAG--- 822
OsCDKB2      ACGACGTGAACAAGG---AGCTCTACTGA 909
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PAM sequence is shown in cyan and 20bp target sequence is shown in red.

Supplementary Figure S3. Mutations in CDKAs and CDKBs in regenerated plants obtained from pZH_OsCas9, pZK_sgCDKB2 transformed calli #5,11,12,13.

CDKB2
CGAGAAGCTGGAGAAGGT**TCGGGGAGGGGACGTACGGG**AAGGTGTACAAGGC CGG WT
CGAGAAGCTGGAGAAGGT**TCGGGGAGGGGAC**-TACGGGAAGGTGTACAAGGC CGG (-1) #5-1, 3, #11-2, 3, 4, #12-1
CGAGAAGCTGGAGAAGGT**TCGGGGAGGGGA**--TACGGGAAGGTGTACAAGGC CGG (-2) #11-2
CGAGAAGCTGGAGAAGGT**TCGGGGAGGGGACGA**TACGGGAAGGTGTACAAGGC CGG (+1) #5-4, 6, 7, 10, #11-1

CDKA2
CGAGAAGGTGGAGAAG**ATCGGGGAGGGGACGTACGGG**TGGTGTACAAGGGCAAG WT
CGAGAAGGTGGAGAAGAT**CGGGGAGGGGACG**----GGGTGGTGTACAAGGGCAAG (-4) #11-3, 4
CGAGAAGGTGGAGAAGAT**CGGGGAGGGGAC**-TACGGGGTGGTGTACAAGGGCAAG (-1) #5-1, 3, 4, #11-2, 3, 5, 6, #12-1
CGAGAAGGTGGAGAAGAT**CGGGGAGGGGACG**-ACGGGGTGGTGTACAAGGGCAAG (-1) #5-5
CGAGAAGGTGGAGAAGAT**CGGGGAGGGGACGA**TACGGGGTGGTGTACAAGGGCAAG (+1) #5-6, 7, #11-1

CDKB1
CGAGAAGCTGGAGAAGGT**GGGGGAAGGGACGTACGGG**AAGGTGTACAAGGC WT
CGAGAAGCTGGAGAAGGTGGGGGAAGGGAC**GTACGGGAAGGTGTACAAGGC** (+1) #5-1
CGAGAAGCTGGAGAAGGTGGGGGAAGGGAC-TACGGGAAGGTGTACAAGGC (-1) #11-2, 3, 4
CGAGAAGCTGGAGAAGGTGGGGGAAGGGAC**T**TACGGGAAGGTGTACAAGGC (+1) #13-1
CGAGAAGCTGGAGAAGGTGGGGGAAGGG-----TGTACAAGGC (-13) #5-3
CGAGAAGCTGGAGAAGGTGGGGGAAGGGACGTACGGGAAG**GG**TACAAGGC (m2) #5-4

Representative sequences of mutant alleles identified from Cas9, sgRNA expressed calli. The wild type sequence is shown at the top with PAM sequence highlighted in cyan and the target sequence in red. Dash, deleted bases. The net change in length is to the right of each sequence (+, insertion; -, deletion).

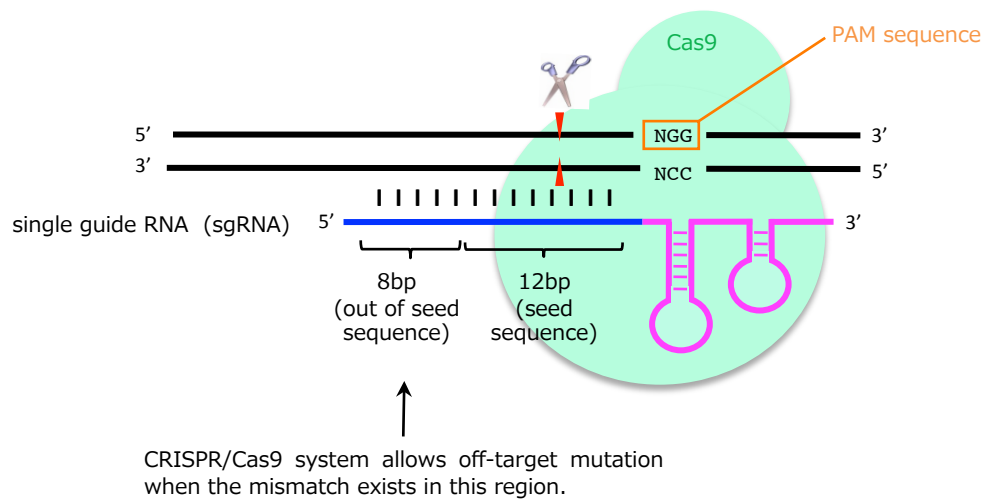
Supplementary Figure S4. Potential off target sites predicted by CRISPR-P.

	AGGTCGGGGAGGGGACGTACGGG	On-target	(Chr.8 CDKB2)
1	AGATCGGGGAGGGGACGTACGGG	Detected	(Chr.2 CDKA2)
2	AGGTGGGGAGGGGACGTACGGG	Detected	(Chr.1 CDKB1)
3	CGGCGGGCGTGGGGACGTACAG	No-detected	(Chr.2)
4	GGTCGGGGAGGGGACGTTCGGG		(Chr.1)
5	GGCAGGGCAGGGGACGTACGGG	No-detected	(Chr.2)
6	GGGGAGGAGGGGACGTACAGG		(Chr.3)
7	AGGCGTGAAGGGGACGTACGGG		(Chr.7)
8	AGGGAAGGAGGAGACGTACAGG		(Chr.3)
9	AGGACGAGGAGGGACGTACGGG	No-detected	(Chr.3)
10	AGATTGGGGAGGGACGTACGGG	No-detected	(Chr.3 CDKA1)

BsiWI

The top 10 off-target sites predicted by CRISPR-P are listed. CDKA2, CDKB1 and CDKA1 are ranked as 1st, 2nd and 10th. Among 6 analyzed off-target candidates, mutations were detected in only top two candidates, CDKA2 and CDKB1.

Supplementary Figure S5. Acceptable mismatch in CRISPR/Cas9 system.



CRISPR/Cas9 system allows off-target mutation when mismatches exist in out of seed sequence.