Supplementary data

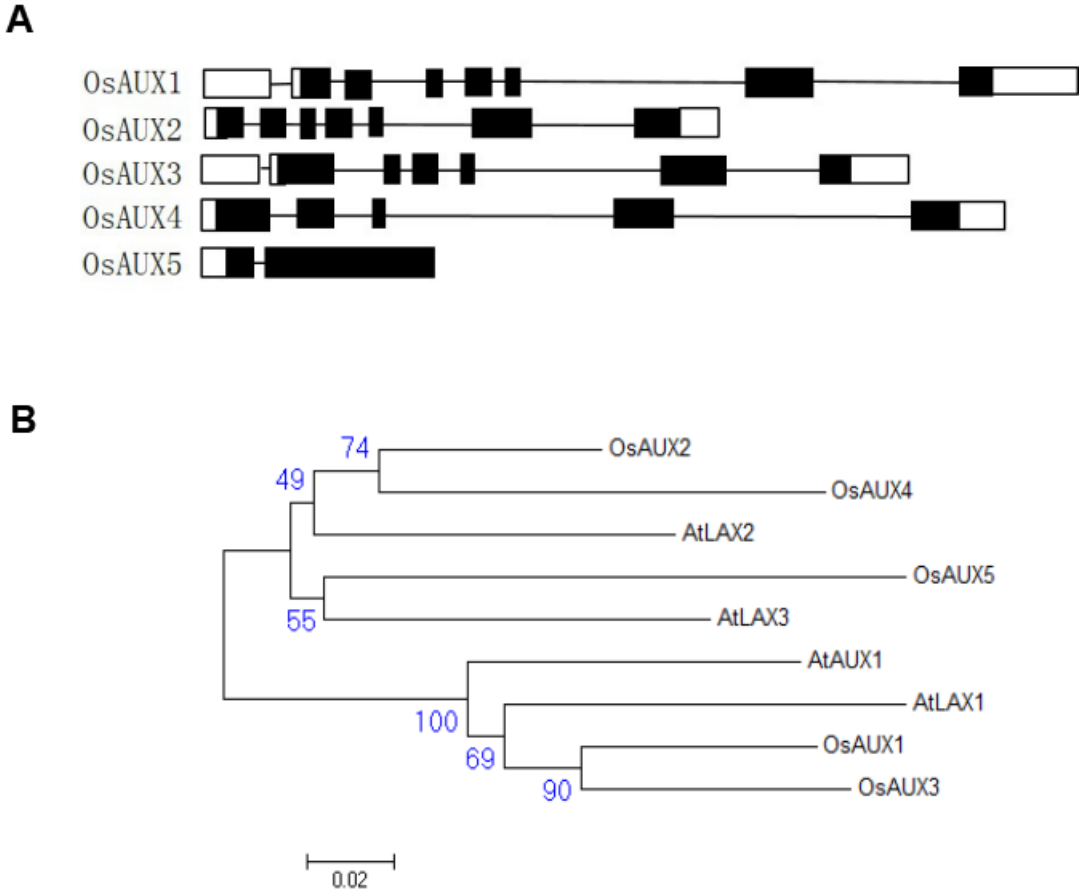
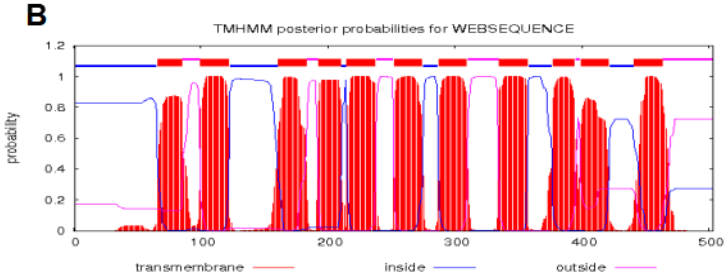


Figure S1. Gene structure and phylogenetic tree of *OsAUX1/LAX* family.
(A) Analysis of gene structure of *OsAUX1/LAX* family. White box represents non-translation area, black box represents exon, and black line represents intron.
(B) Analysis of phylogenetic tree of AUX1/LAX family between rice and Arabidopsis thaliana. Use MEGA 5 software to construct phylogenetic tree. Bootstrap test using 1000 repetitions. Os, *Oryza sativa*; At, *Arabidopsis thaliana*.

A

	OsAUX3
OsAUX1	84. 15%
OsAUX2	71. 37%
OsAUX3	100%
OsAUX4	70. 58%
OsAUX5	72. 71%

B



C

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OsAUX2     -MGSAADGSLANEKAPAETWGWGRYVEMEQDGGG--------PSTAKS---RLSGLL 45
AtLAX2     -------MENGEKAAETVVVGNYVEMEKDGK---------ALDIKS---KLSDMF 36
OsAUX4     MASGSSGGGYADEKGPGAATMQALGLQQQHGGGGEVEEESSEMGEKTAART---RLSGLL 57
OsAUX5     -------MASEK---VETIVAGNYVEMERBGAATAGBGWGGAAAASGRRRGKLAVSSLF 49
AtLAX3     -------MAAEK---IETVVAGNYLEMEREEENISGN-----KKSSTKT---KLSNFF 40
OsAUX1     ------MVPR-EQAEEAIVADSNGKEEEVG-VMGVSAGEHGADDHHGGGGKFSMKNLL 50
OsAUX3     ------MVPAGDQAEEAIVADAGKEEAEVRAAMGVEQ---------DGKFSMTSLL 41
AtAUX1     ---------MSBGVEAIVANDNGTDQVNGNRTGKDNEE-----HDGSTGSNLSNFL 42
AtLAX1     -------MSGEKQAEESIVVSGDEEVAGRKVEDSAAEEDID----GNGGNGFSMKSFL 47
                        .    :                                  :..::

OsAUX2     WHGGSAYDAWFSCASN---------------QVAQVLLTLPYSFSQLGMLSGIL 84
AtLAX2     WHGGSAYDAWFSCASN---------------QVAQVLLTLPYSFSQLGMLSGIL 75
OsAUX4     WHGGSAYDAWFSCASN---------------QVAQVLLTLPYSFAQLGMASGLL 96
OsAUX5     WHGGSVYDAWFSCASN---------------QVAQVLLTLPYSFSQLGMASGVA 88
AtLAX3     WHGGSVYDAWFSCASN---------------QVAQVLLTLPYSFSQLGMMSGIL 79
OsAUX1     WHGGSVWDAWFSCASN---------------QVAQVLLTLPYSFSQLGMLSGVL 89
OsAUX3     WHGGSVWDAWFSCASNQVRPTTNDLVMPLAHISFGILQVAQVLLTLPYSFSQLGMLSGLL 101
AtAUX1     WHGGSVWDAWFSCASN---------------QVAQVLLTLPYSFSQLGMLSGIV 81
AtLAX1     WHGGSAWDAWFSCASN---------------QVAQVLLTLPYSXSQLGMLSGIL 86
           *****.:*********                ************ :**** **:

OsAUX2     FQLFYGLLGSWTAYLISILYVEYRTRKER-EKVDFRNHVIQWFEVLDGLLGRHWRNVGLA 143
AtLAX2     FQLFYGILGSWTAYLISILYVEYRTRKER-EKVNFRNHVIQWFEVLDGLLGKHWRNVGLA 134
OsAUX4     FQLFYGLLGSWTAYLISILYLEYRTRKER-DKVDFRNHVIQWFEVLDGLLGRHWRNVGLA 155
OsAUX5     FQVFYGLMGSWTAYLISVLYVEYRTRRER-DKVDFRNHVIQWFEVLDGLLGRHWRNAGLL 147
AtLAX3     FQLFYGLMGSWTAYLISVLYVEYRTRKER-EKFDFRNHVIQWFEVLDGLLGKHWRNLGLI 138
OsAUX1     LQLFYGFMGSWTAYLISVLYVEYRSRKEK-BGVSFKNHVIQWFEVLDGLLGPYWKAAGLA 148
OsAUX3     LQVFYGLMGSWTAYLISVLYVEYRARKEK-BGVSFKNHVIQWFEVLDGLLGPYWKAAGLA 160
AtAUX1     LQIFYGLLGSWTAYLISVLYVEYRARKEK-BGKSFKNHVIQWFEVLDGLLGSYWKALGLA 140
AtLAX1     LQIFYGLMGSWTAYLISVLYVEYRARMEKQEAKSFKNHVIQWFEVLDGLLGPYWKAAGLA 146
           :*:***::*********:**:***:* *: :  .*:*************** :*:  **

OsAUX2     FNCTFLLFGSVIQLIACASNIYYINDKLDKRTWTYIFGACCATTVFIPSFHNYRIWSFLG 203
AtLAX2     FNCTFLLFGSVIQLIACASNIYYINDNLDKRTWTYIFGACCATTVFIPSFHNYRIWSFLG 194
OsAUX4     FNCTFLLFGSVIQLIGCASNIYYINDHLDKRTWTYIFGACCATTVFIPSFHNYRIWSFLG 215
OsAUX5     FNCTFLLFGSVIQLIACASNIYYINDRLDKRTWTYIFGACCATTVFVPSFHNYRVWSFLG 207
AtLAX3     FNCTFLLFGSVIQLIACASNIYYINDKLDKRTWTYIFGACCATTVFIPSFHNYRIWSFLG 198
OsAUX1     FNCTFLLFGSVIQLIACASNIYYINDRLDKRTWTYIFGACCATTVFIPSFHNYRIWSFLG 208
OsAUX3     FNCTFLLFGSVIQLIACASNIYYINDRLDKRTWTYIFGACCSTTVFIPSFHNYRIWSFLG 220
AtAUX1     FNCTFLLFGSVIQLIACASNIYYINDHLDKRTWTYIFGACCATTVFIPSFHNYRIWSFLG 200
AtLAX1     FNCTFLLFGSVIQLIACASNIYYINDRLDKRTWTYIFGACCATTVFIPSFHNYRIWSFLG 206
           ***************.**********.**************:****:*******:*****

OsAUX2     LVMTTYTAWYLAVASLIHGQVDGVK-HSGPTK-MVLYFTGATNILYTFGGHAVTVEIMHA 261
AtLAX2     LLMTTYTAWYLTIASILHGQVBGVK-HSGPSK-LVLYFTGATNILYTFGGHAVTVEIMHA 252
OsAUX4     LLMTTYTAWYIAVASLIHGQVBGVA-HSGPTS-IVLYFTGATNILYTFGGHAVTVEIMHA 273
OsAUX5     LLMTSYTAWYLTVAAVVHGKVDGAAPRAGPSKTMVLYFTGATNILYTFGGHAVTVEIMHA 267
AtLAX3     LAMTTYTSWYLTIASLLHGQAEDVK-HSGPTT-MVLYFTGATNILYTFGGHAVTVEIMHA 256
OsAUX1     LGMTTYTAWYLAIAALLNGQABGIT-HTGPTK-LVLYFTGATNILYTFGGHAVTVEIMHA 266
OsAUX3     LGMTTYTAWYLAIAAAVHGQVDGVT-HSGPSK-MVLYFTGATNILYTFGGHAVTVEIMHA 278
AtAUX1     LGMTTYTAWYLAIASIIHGQABGVK-HSGPTK-LVLYFTGATNILYTFGGHAVTVEIMHA 258
AtLAX1     LGMTTYTAWYLTIASFLHGQABGVT-HSGPTK-LVLYFTGATNILYTFGGHAVTVEIMHA 264
           * **:**:**:::*: ::*:.:.   ::**:. :*************************
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OsAUX2          MWRPQKFKAIYLMATLYVLTLTLPSAASVYWAFGDELLTHSNALALLPRTAFRDAAVVLM 321
AtLAX2          MWKPQKFKSIYLFATLYVLTLTLPSASAVYWAFGDLLLNHSNAFALLPKNLYRDFAVVLM 312
OsAUX4          MWRPQKFKAIYLLATVYVLTLTLPSASAAYWAFGDALLTHSNALALLPRTPWRDAAVVLM 333
OsAUX5          MWRPRRFKMIYLAATAYVLTLTLPSAAAMYWAFGDALLDHSNAFALLPRTPWRDAAVVLM 327
AtLAX3          MWKPQKFKAIYLLATIYVLTLTLPSASAVYWAFGDKLLTHSNALSLLPKTGFRDTAVILM 316
OsAUX1          MWKPAKFKYIYLLATLYVFTLTLPSASAMYWAFGDELLTHSNAFSLLPKTGWRDAAVILM 326
OsAUX3          MWKPQKFKYIYLVATLYVFTLTLPSASAMYWAFGDALLTHSNAFSLLPRSGWRDAAVILM 338
AtAUX1          MWKPQKFKYIYLMATLYVFTLTIPSAAAVYWAFGDALLDHSNAFSLMPKNAWRDAAVILM 318
AtLAX1          MWKPRKFKSIYLMATLYVFTLTLPSASALYWAFGDQLLNHSNAFSLLPKTRFRDTAVILM 324
                **:* :** *** ** **:***:***:: ****** ** ****::*:*:. :** **:**

OsAUX2          LIHQFITFGFACTPLYFVWEKLIGLHDCRSLFKRAAARLPVVVPIWFLAIIFPFFGPINS 381
AtLAX2          LIHQFITFGFACTPLYFVWEKLIGMHECRSMCKRAAARLPVVIPIWFLAIIFPFFGPINS 372
OsAUX4          LIHQFITFGFACTPLYFVWEKLVGLHGCPSLCKRAAARLPVVLPIWFLAIIFPFFGPINS 393
OsAUX5          LIHQFITFGFACTPLYFVWEKAIGVHGGAGVLRRAAARLPVVLPIWFLAVIFPFFGPINS 387
AtLAX3          LIHQFITFGFASTPLYFVWEKLIGVHETKSMFKRAMARLPVVVPIWFLAIIFPFFGPINS 376
OsAUX1          LIHQFITFGFACTPLYFVWEKVIGMHDTKSICLRALARLPIVVPIWFLAIIFPFFGPINS 386
OsAUX3          LIHQFITFGFACTPLYFVWEKAIGMHGTRSVLTRALARLPIVVPIWFLAIIFPFFGPINS 398
AtAUX1          LIHQFITFGFACTPLYFVWEKVIGMHDTKSICLRALARLPVVIPIWFLAIIFPFFGPINS 378
AtLAX1          LIHQFITFGFACTPLYFVWEKAIGMHHTKSLCLRALVRLP-CVPIWFWPIIFPFS-AINS 382
                **********.******** :*:*  .:  ** .***  :**** .:****  .***

OsAUX2          AVGSLLVSFTVYIIPALAHMITFRSAHARENAVEPPPRFVGRWTGTFIINAFVVAWVLVV 441
AtLAX2          TVGSLLVSFTVYIIPALAHIFTFRSSAARENAVEQPPRFLGRWTGAFTINAFIVVWVFIV 432
OsAUX4          AVGSLLVSFTVYIIPSLAYMVTFRSPQSRQNAVERPPRFAGGWTGAYVINSFVVAWVLVV 453
OsAUX5          TVGSFLVSFTVYIIPAMAHMATFAPAAARENAVEPPPRALGGWPGTFAANCFVVAWVLVV 447
AtLAX3          AVGSLLVSFTVYIIPALAHMLTFAPAPSRENAVERPPRVVGGWMGTYCINIFVVVWVFVV 436
OsAUX1          AVGALLVSFTVYIIPALAHILTYRTASARMNAAEKPPFFLPSWTGMFVLNMFIVVWVLVV 446
OsAUX3          AVGALLVSFTVYIIPSLSHILTYRSASARLNAAEKPPFFLPSWSGMFVVNVFVVAWVLVV 458
AtAUX1          AVGALLVSFTVYIIPSLAHMLTYRSASARQNAAEKPPFFMPSWTAMYVLNAFVVVWVLIV 438
AtLAX1          AVGALLVTFTVYIIPALAHMLTYRTASA-AERAEKPPFFIPSWAGVYVINAFIVVWVLVL 441
                :**::**:******::::: *: .. :  : .* **    * . :  * *:*.**:::

OsAUX2          GFGFGGWASMTNFVRQIDTFGLFTKCYQCPPPPLPPAGAAPNATW--PPFPATPFNATTA 499
AtLAX2          GFGFGGWASMINFVHQIDTFGLFTKCYQCPPPVMVSP--------PPISHPHFN---- 478
OsAUX4          GFGFGGWASITNFVHQVDTFGLFAKCYQCPPHPAAAALSPPGAIAPAPASMLPPFNSTAA 513
OsAUX5          GFGFGGWASTVNFVRQVDTFGLFTKCYQCPPRH-------------------------- 480
AtLAX3          GFGFGGWASMVNFVRQIDTFGLFTKCYQCPPHKP------------------------- 470
OsAUX1          GFGLGGWASMVNFIRQIDTFGLFAKCYQCPKPAPALAQSPVPLPHH------------- 492
OsAUX3          GFGLGGWASVTNFIKQIDTFGLFAKCYQCPPRAHAGAPLPAPPRH-------------- 503
AtAUX1          GFGFGGWASVTNFVRQVDTFGLFAKCYQCKP-AAAAAHAPVSALHHRL----------- 485
AtLAX1          GFGFGGWASMTNFIRQIDTFGLFAKCYQCKP---PPAPIAAGAHHRR------------ 485
                ***:*****  **::*:******:*****

OsAUX2          GLAPAPAPSPAHF--------FGRHHRHHSHGL 524
AtLAX2          ----------------------HTHGL 483
OsAUX4          GIFAAPVPSPAPAPAPMHFVLGHHHHHRHHRHGL 547
OsAUX5          ---------------------------
AtLAX3          ---------------------------
OsAUX1          ---------------------------
OsAUX3          ---------------------------
AtAUX1          ---------------------------
AtLAX1          ---------------------------
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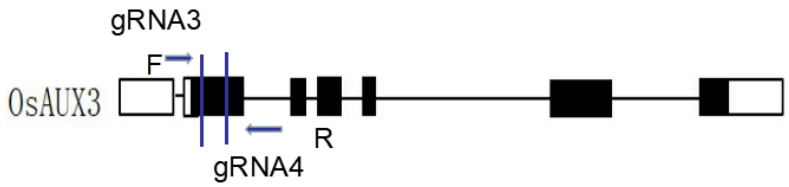
Figure S2. Protein structure of OsAUX3.

(A) Similarity analysis of OsAUX1/LAX family protein. Clustalw2 software was used to analyze the similarity of OsAUX1/LAX proteins.

(B) Prediction of transmembrane regions of OsAUX3 protein. The red rectangle represents transmembrane, the blue line represents inside, and the pink line represents outside.

(C) Protein alignment analysis of AUX1/LAX family in the rice and Arabidopsis. The red box indicates the specific amino sequence of OsAUX3 different to the other member.

A



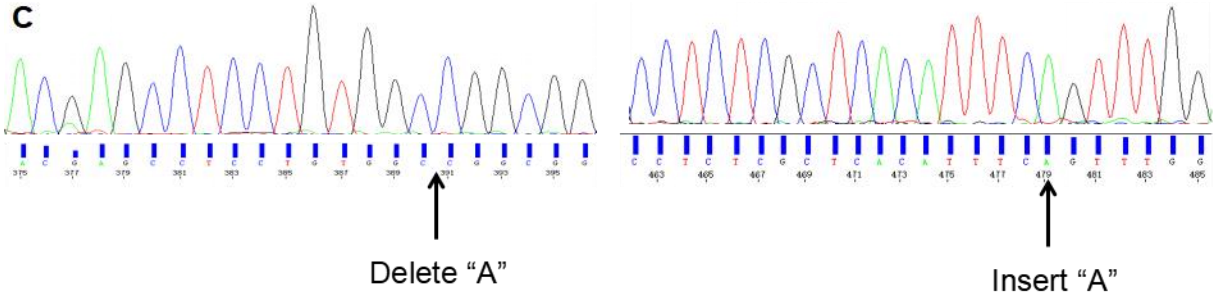
B

5'.....CATGACGAGCCTCCTGTGGCACGGCGG.....CATGCCTCTCGCTCACATTTCGTTTGG.....3'

gRNA3 gRNA4

Gene ID	Gene architecture
PTG3	tRNA-gRNA3-tRNA-gRNA4

C

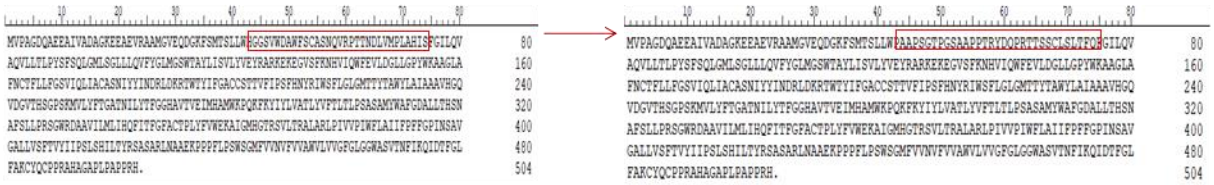


RNA

WT/HY 5'.....(121bp)CTGTGGCACGGCGG..... （220bp）TCGTTTGG（227bp）.....3'

osaux3-1 *5'*.....(121bp)CTGTGGC-CGGCGG..... （220bp）TCAGTTTGG（227bp）.....3'

Protein



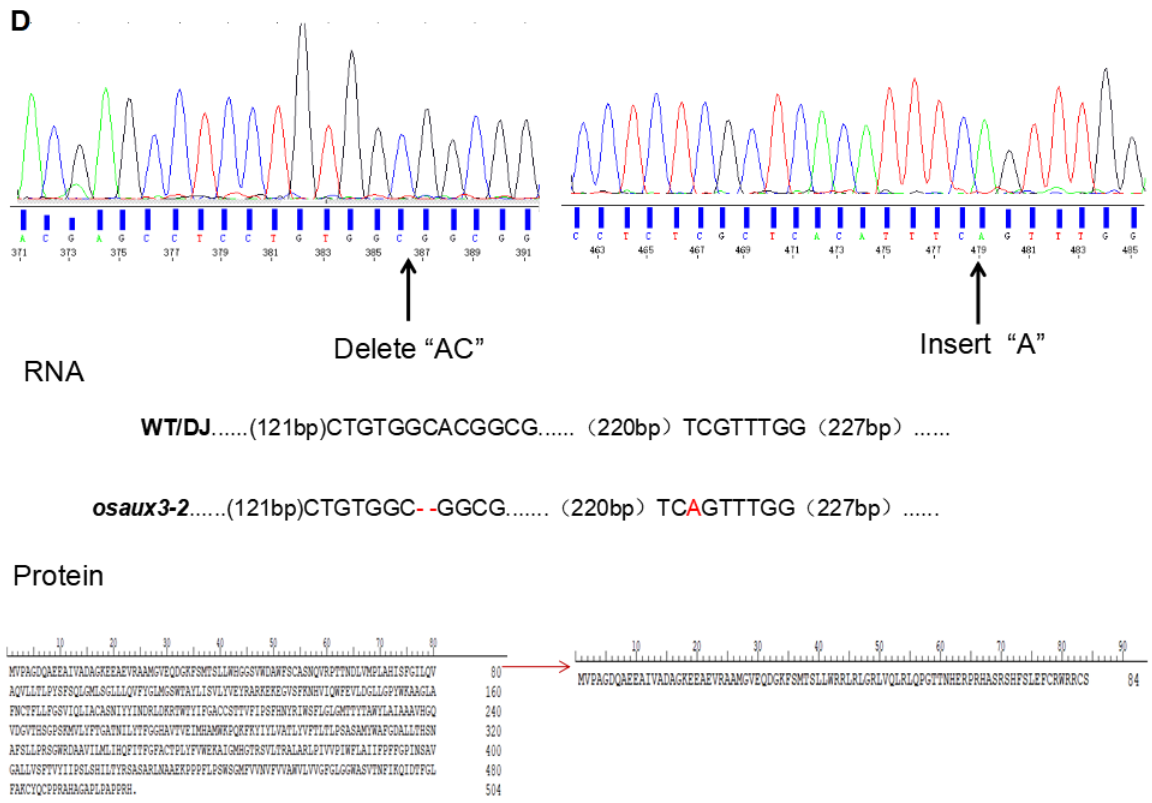


Figure S3. Analysis of the targeted mutation of OsAUX3 gene in transgenic rice by CRISPR/CAS9.

(A) Schematic depiction of OsAUX3 locus，black box represents exon. The targeting sites of gRNA3 and gRNA4, and the location of primers (F and R) for genotyping are indicated with blue line and arrow.

(B) Targeting sequences of gRNA3 and gRNA4 are highlighted with red color and gene architecture of PTG3 was drawn with three-line table.

(C) The positions of gene editing in *osaux3-1*. On DNA sequence, for targeting sequences of gRNA3, one adenine (A) located at 128bp of open reading frame of *OsAUX3* was deleted, and for targeting sequences of gRNA4, one A was inserted within 221bp and 222bp, which was shown with black arrow. On protein sequence, the sequence of amino acids was changed in the special peptide sequence of OsAUX3 from 43-75 amino acids.

(D) The positions of gene editing in *osaux3-2*. On the DNA sequence, for targeting sequences of gRNA3, A and C located 128-129bp of open reading frame of *OsAUX3* was deleted, and for targeting sequences of gRNA4, one A was inserted within 221bp and 222bp, which was shown with black arrow. On protein sequence, the open reading frame of *OsAUX3* was terminated in advance.

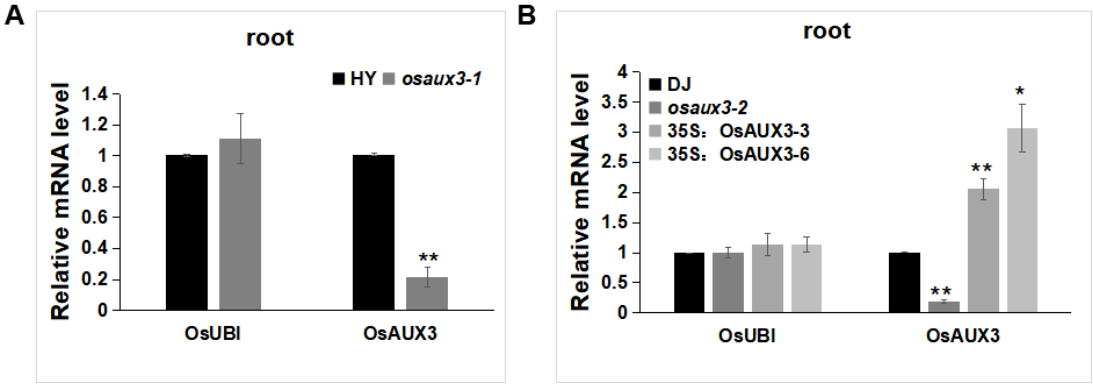


Figure S4: qRT-PCR analysis of *OsAUX3* expression

(A) qRT-PCR analysis of *OsAUX3* expression. qRT-PCR experiments were analyzed using three independent biological replicas. *OsACTIN* and *OsUBI* were used as internal control. ** indicate significant differences compared with WT/HY ($P < 0.01$) in Student's t-tests.

(B)Relative mRNA level of *OsAUX3* in WT/DJ, *osaux3-2* and *OsAUX3-overexpression* lines. qRT-PCR experiments were analyzed using three independent biological replicas. *OsACTIN* and *OsUBI* were used as internal control. Asterisks indicate significant differences compared with WT/DJ, respectively (**$P < 0.01$; *$P < 0.05$; t-test).

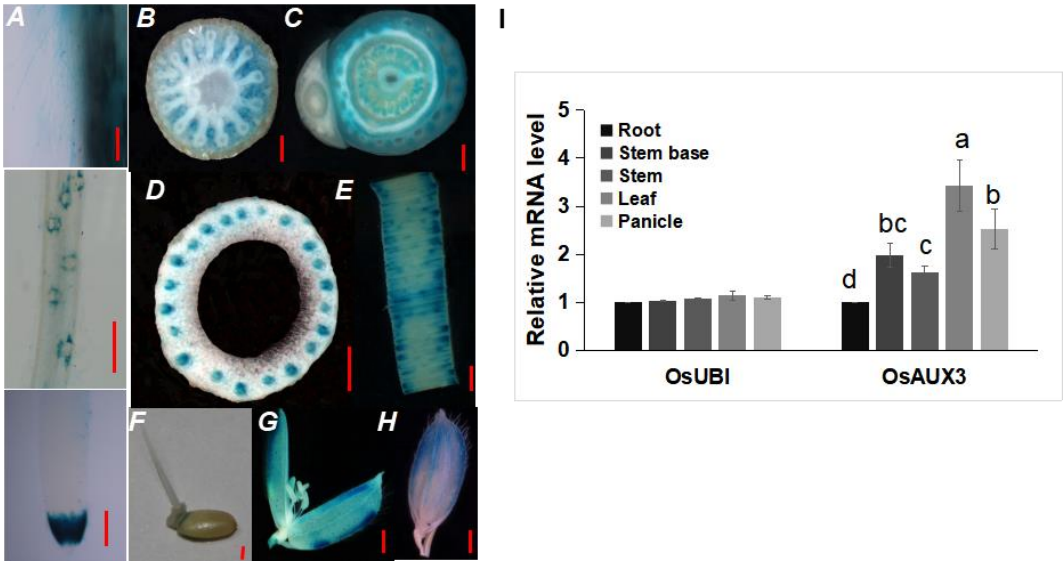


Figure S5. Expression pattern of the *OsAUX3* gene.

(A-H)The expression pattern of OsAUX3. β-glucuronidase (GUS) staining was examined in *ProOsAUX3-GUS* transgenic rice seedlings. Primary root (A), stem node (B), stem base (C), stem (D), leaf(E), seed (F), flower(G), glume (H), bar=500 μm.

(I) Quantitative reverse transcription PCR of *OsAUX3* in various tissues. Total RNA was extracted from the root, stem base, stem, mature leaf and panicle of WT/DJ. Experiments were analyzed using three independent biological replicas. *OsACTIN* and *OsUBI* were used as internal control. Columns with different letters indicate significant differences (Duncan's test, $p < 0.05$).

Table S1. Primers for vector construction used in this study.

Gene	Primers sequence (from 5’ to 3’)
OsAUX3gRNA3(MPK1)-FP	TAGGTCTCCCCTGTGGCACGGgttttagagctagaa
OsAUX3gRNA3(MPK1)-RP	ATGGTCTCACAGGAGGCTCGTtgcaccagccgggaa
OsAUX3gRNA4(MPK1)-FP	TAGGTCTCCTCACATTTCGTTgttttagagctagaa
OsAUX3gRNA4(MPK1)-RP	CGGGTCTCAGTGAGCGAGAGGtgcaccagccggg
L5AD5-F	CG GGTCTC A GGCA GGATG GGCAGTCTG GGCAACAAAGCACCAGTGG
L3AD5-R	TA GGTCTC C AAAC GGATG AGCGACAGC AAACAAAAAAAAAA GCACCGACTCG
promoter u3	TGGGTACGTTGGAAACCACG
pUBI10-RP	GTTTGTTGGTCGCCGTTAGG
OsAUX3(Crispr)-FP	GTCTCCTCACTCTCGACTAC
OsAUX3(Crispr)-RP	TGACGCGAAGATGTGCAAGG
35S:OsAUX3-GFP-FP	GAGCTCATGGTGCCGGCCGGCG
35S:OsAUX3-GFP-RP	TCTAGAGTGGCGCGGCGGAG
OsAUX3pro-OsAUX3-GFP-FP	gaattccgtggtttgctgggggcg
OsAUX3pro-OsAUX3-GFP-RP	gagctccctcctgtcctccccggt
OsAUX3pro-GUS--FP	AAGCTTACACGTAGGCGCCACCATGG
OsAUX3pro-GUS--RP	GGATCCCCTCCTGTCCTCCCCGGT

Table S2. qRT-PCR Primers used in this study.

Gene	Primers sequence (from 5’ to 3’)
Actin-RT-FP	TCAGCAACTGGGATGATATGGAG
Actin-RT-RP	GCCGTTGTGGTGAATGAGTAAC
OsUBI-RT-FP	AACCAGCTGAGGCCCAAGA
OsUBI-RT-RP	ACGATTGATTTAACCAGTCCATGA
OsAUX3-RT-FP	TTGGAATTTTGCAGGTGGCG
OsAUX3-RT-RP	ACCACTGGATGACGTGGTTC
OsCYCB2;2-RT-FP	CTACACTGCTCAGTGCACCA
OsCYCB2;2-RT-RP	TTACAGTGCCACGCTCTTGA
OsCYCD4;1-RT-FP	CTGCCTCAGCTACAGGAGTG
OsCYCD4;1-RT-RP	ACCGGGGAGCTATCATGTTC
OsCYCU4;3-RT-FP	GGTGGACTTCCTCTTTGGCA
OsCYCU4;3-RT-RP	GCTGCTCCAGGTAGGTCATC
OsCDKC;1-RT-FP	ACAAATGCGTCAAGCCGATG
OsCDKC;1-RT-RP	ATGGGGCTGTCCTGTTTGTT
OsCDKC;3-RT-FP	GCCATTCACTAGAGACGGAAGT
OsCDKC;3-RT-RP	CATGTGCGGAAATCCTCTCAGTA
OsCKL;10-RT-FP	GGCGCTAAGAAGAGCAGGAT
OsCKL;10-RT-RP	TCTCGACGTGATTCTTGCCC
OsALS1-RT-FP	TCGTCAGTCTCTGCCTTGTC
OsALS1-RT-RP	TCCCCATCATTTTCATTTGT
OsMGT1-RT-FP	AGGAAGCTGTCGTTCCAAGG
OsMGT1-RT-RP	GGCCACTCTCTGTCTCATCG
OsNrat1-RT-FP	CAGGTCATCATGCAGGGGTT
OsNrat1-RT-RP	CGATGACTCGGGTGATGAGG
OsART1-RT-FP	ATGGATTTCGGATTCGGGCA
OsART1-RT-RP	CCTGATGATCACCCGCGAAT