Supplementary Materials

Table S1. Primer sequences used for synthesizing gRNA spacers and genotyping CRISPR-edited mutants.

| Name	Sequence 5′→3′	Purpose
gRNAs-*qRA2*	gtcgctgtgccgctccaccaagg	CRISPR/CAS9
qRA2-F1	CCACAGATCTTCTTCCTTCC	Screening of lines
qRA2-R1	GGTATCCGAGATAGCTGATGG	Screening of lines
*qRA2*cDS-R	ATGGGTTCTCTGGGAGCAG	Gene amplification
*qRA2*cDS-F	CTACGACTCTTTGGCCAGGA	Gene amplification
qRA2-qRT-PCR-F	CCGTCACTACCGCCTCCTCA	RT-qPCR
qRA2-qRT-PCR-R	CGGCGTGTCAAGGTATCCATTC	RT-qPCR
UBIQUITIN-F	AACCAGCTGAGGCCCAAGA	Reference gene
UBIQUITIN-R	ACGATTGATTTAACCAGTCCATGA	Reference gene
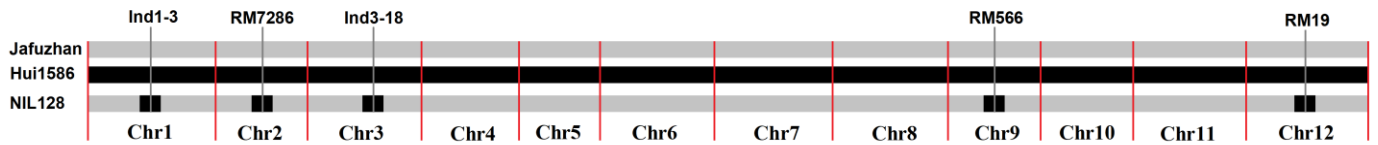
Figure S1. Analysis of the genetic background of NIL128. NIL128 basically recovered the genetic background of the recipient parents except for 5 loci.

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Nip    ATGGGTTCTCTGGGAGCAGCAGGTAAGCCGCACGCCGTGTGCATGCCGTACCCGGCGCAGGGGCACATCACCCCGATGCTGAACGTGGCGAAGCTGCTCCACGCCCGCGGCTTCGACGTC    120
qRA2   ATGGGTTCTCTGGGAGCAGCAGGTAAGCCGCACGCCGTGTGCATGCCGTACCCGGCGCAGGGGCACATCACCCCGATGCTGAACGTGGCGAAGCTGCTCCACGCCCGCGGCTTCGACGTC    120
qpa2   ACGTTCTGGGAGCAGCAGGTAAGCCGCACGCCGTGTGCATGCCGTACCCGGCGCAGGGGCACATCACCCCGATGCTGAACGTGGCGAAGCTGCTCCACGCCCGCGGCTTCGACGTC       120
Consensatgggttctctgggagcagcaggtaagccgcacgccgtgtgcatgccgtacccggcgcaggggcacatcaccccgatgctgaacgtggcgaagctgctccacgcccgcggcttcgacgtc

Nip    ACGTTCGTGAACACCGAGTACAACCACGCCCGCCTCGTCCGCACCCGCGGCGAGGCCGCGGTGGCGGGCGCGCCGGGCTTCCGGTTCGCCACCATCCCCGACGGCCTGCCGCCGTCGGAC    240
qRA2   ACGTTCGTGAACACCGAGTACAACCACGCCCGCCTCGTCCGCACCCGCGGCGAGGCCGCGGTGGCGGGCGCGCCGGGCTTCCGGTTCGCCACCATCCCCGACGGCCTGCCGCCGTCGGAC    240
qpa2   ACGTTCGTGAACACCGAGTACAACCACGCCCGCCTCGTCCGCACCCGCGGCGAGGCCGCGGTGGCGGGCGCGCCGGGCTTCCGGTTCGCCACCATCCCCGACGGCCTGCCGCCGTCGGAC    240
Consensacgttcgtgaacaccgagtacaaccacgcccgcctcgtccgcacccgcggcgaggccgcggtggcgggcgcgccgggcttccggttcgccaccatccccgacggcctgccgccgtcggac

Nip    GACGACGACGTCACGCAGGACATCCCGTCGCTGTGCCGCTCCACCAAGGAGACCTGCCTTGCCCCCTTCCGCCGCCTCCTCGCCCAGCTGAATGACCCCGCCACGGGCCACCCGCCCGTC    360
qRA2   GACGACGACGTCACGCAGGACATCCCGTCGCTGTGCCGCTCCACCAAGGAGACCTGCCTTGCCCCCTTCCGCCGCCTCCTCGCCCAGCTGAATGACCCCGCCACGGGCCACCCGCCCGTC    360
qpa2   GACGACGACGTCACGCAGGACATCCCGTCGCTGTGCCGCTCCACCAAGGAGACCTGCCTTGCCCCCTTCCGCCGCCTCCTCGCCCAGCTGAATGACCCCGCCACGGGCCACCCGCCCGTC    360
Consensgacgacgacgtcacgcaggacatcccgtcgctgtgccgctccaccaaggagacctgccttgccccccttccgccgcctcctcgcccagctgaatgaccccgccacgggccacccgcccgtc

Nip    ACCTGCGTCGTCTCCGACGTCGTCATGGGTTTCTCCATGGCTGCCGCCAAGGAGCTCGGCCTCCCCTACGTCCAGCTCTGGACAGCCAGCTCCATCAGCTATCTCGGATACCGTCACTAC    480
qRA2   ACCTGCGTCGTCTCCGACGTCGTCATGGGTTTCTCCATGGCTGCCGCCAAGGAGCTCGGCCTCCCCTACGTCCAGCTCTGGACAGCCAGCTCCATCAGCTATCTCGGATACCGTCACTAC    480
qra2   ACCTGCGTCGTCTCCGACGTCGTCATGGGTTTCTCCATGGCTGCCGCCAAGGAGCTCGGCCTCCCCTACGTCCAGCTCTGGACAGCCAGCTCCATCAGCTATCTCGGATACCGTCACTAC    480
Consensacctgcgtcgtctccgacgtcgtcatgggtttctccatggctgccgccaaggagctcggcctcccctacgtccagctctggacagccagctccatcagctatctcggataccgtcactac

Nip    CGCCTCCTCATGGGAACGTGGCCTTGCCCCACTCAAAGATGTCGATCAGCTGACGAATGGATACCTTGACACGCCGGTGGAAGACGTGCCGGGGCTGAGGAACATGAGGATCAAGGACTTC    600
qRA2   CGCCTCCTCATGGGAACGTGGCCTTGCCCCACTCAAAGATGTCGATCAGCTGACGAATGGATACCTTGACACGCCGGTGGAAGACGTGCCGGGGCTGAGGAACATGAGGATCAAGGACTTC    600
qra2   CGCCTCCTCATGTAACGTGGCCTTGCCCCACTCAAAGATGTCGATCAGCTGACGAATGGATACCTTGACACGCCGGTGGAAGACGTGCCGGGGCTGAGGAACATGAGGATCAAGGACTTC    600
Consenscgcctcctcatg aacgtggccttgccccactcaaagatgtcgatcagctgacgaatggataccttgacacgccggtggaagacgtgccggggctgaggaacatgaggatcaaggacttc

Nip    CCGAGCTTCATACACACGACGAACCCGGAGGAGTACATGGTAGGCTACGTCATCGAGGAGACGGAGCGCTGCAAGGATGCCTCGGCCATCATCGTCAACTCCTTCGGCGACCTCGAGGGT    720
qRA2   CCGAGCTTCATACACACGACGAACCCGGAGGAGTACATGGTAGGCTACGTCATCGAGGAGACGGAGCGCTGCAAGGATGCCTCGGCCATCATCGTCAACTCCTTCGGCGACCTCGAGGGT    720
qra2   CCGAGCTTCATACACACGACGAACCCGGAGGAGTACATGGTAGGCTACGTCATCGAGGAGACGGAGCGCTGCAAGGATGCCTCGGCCATCATCGTCAACTCCTTCGGCGACCTCGAGGGT    720
Consensccgagcttcatacacacgacgaacccggaggagtacatggtaggctacgtcatcgaggagacggagcgctgcaaggatgcctcggccatcatcgtcaactccttcggcgacctcgagggt

Nip    GAGGCGGTGGCGGCCATGGAGGCGCTCGGCTTGCCCAAGGTGTACACGCTCGGGCCGCTGCCGCTGGTGGCGCGCAAGGACCCGCCCTCGCCGCGCGGCGCTCGTCCATCAGGCTCAGCCTG    840
qRA2   GAGGCGGTGGCGGCCATGGAGGCGCTCGGCTTGCCCAAGGTGTACACGCTCGGGCCGCTGCCGCTGGTGGCGCGCAAGGACCCGCCCTCGCCGCGCGGCGCTCGTCCATCAGGCTCAGCCTG    840
qra2   GAGGCGGTGGCGGCCATGGAGGCGCTCGGCTTGCCCAAGGTGTACACGCTCGGGCCGCTGCCGCTGGTGGCGCGCAAGGACCCGCCCTCGCCGCGCGGCGCTCGTCCATCAGGCTCAGCCTG    840
Consensgaggcggtggcggccatggaggcgctcggcttgcccaaggtgtacacgctcgggccgctgccgctggtggcgcgcaaggacccgccctcgccgcggcgcgctcgtccatcaggctcagcctg

Nip    TGGAAGGAGCAGGAGGAGTGCCTTCAGTGGCTCGACGGCAAGGAGGCTGGCTCCGTCGTGTACGTCAACTTCGGCAGCATCACCGTCATGACCAACGAGCAGCTGGTGGAGTTCGCGTGG    960
qRA2   TGGAAGGAGCAGGAGGAGTGCCTTCAGTGGCTCGACGGCAAGGAGGCTGGCTCCGTCGTGTACGTCAACTTCGGCAGCATCACCGTCATGACCAACGAGCAGCTGGTGGAGTTCGCGTGG    960
qra2   TGGAAGGAGCAGGAGGAGTGCCTTCAGTGGCTCGACGGCAAGGAGGCTGGCTCCGTCGTGTACGTCAACTTCGGCAGCATCACCGTCATGACCAACGAGCAGCTGGTGGAGTTCGCGTGG    960
Consenstggaaggagcaggaggagtgccttcagtggctcgacggcaaggaggctggctccgtcgtgtacgtcaacttcggcagcatcaccgtcatgaccaacgagcagctggtggagttcgcgtgg

Nip    GGGCTGGCCAACAGCGGCAGGGAGTTCCTGTGGATCGTCCGGCGCGACCTCGTCAAGGGCGACACGGCCGTGCTGCCGCCGCGGAGTTCCTGGCCGAGACGGCGGAGCGCGGCCTCATGGCG    1080
qRA2   GGGCTGGCCAACAGCGGCAGGGAGTTCCTGTGGATCGTCCGGCGCGACCTCGTCAAGGGCGACACGGCCGTGCTGCCGCCGCGGAGTTCCTGGCCGAGACGGCGGAGCGCGGCCTCATGGCG    1080
qpa2   GGGCTGGCCAACAGCGGCAGGGAGTTCCTGTGGATCGTCCGGCGCGACCTCGTCAAGGGCGACACGGCCGTGCTGCCGCCGCGGAGTTCCTGGCCGAGACGGCGGAGCGCGGCCTCATGGCG    1080
Consensgggctggccaacagcggcagggagttcctgtggatcgtccggcgcgacctcgtcaagggcgacacggccgtgctgccgccgcggagttcctggccgagacggcggagcgcggcctcatggcg

Nip    TCCTGGTGCCCGCAGCAGGACGTGCTGAACCACCCCGCCGTGGGCGCGTTCCTGACGCACAGCGGCTGGAACTCGACGCTGGAGAGCTTGGCCGCCGGCGTGCCCGTCATCAGCTGGCCA    1200
qRA2   TCCTGGTGCCCGCAGCAGGACGTGCTGAACCACCCCGCCGTGGGCGCGTTCCTGACGCACAGCGGCTGGAACTCGACGCTGGAGAGCTTGGCCGCCGGCGTGCCCGTCATCAGCTGGCCA    1200
qra2   TCCTGGTGCCCGCAGCAGGACGTGCTGAACCACCCCGCCGTGGGCGCGTTCCTGACGCACAGCGGCTGGAACTCGACGCTGGAGAGCTTGGCCGCCGGCGTGCCCGTCATCAGCTGGCCA    1200
Consenstcctggtgcccgcagcaggacgtgctgaaccaccccgccgtgggcgcgttcctgacgcacagcggctggaactcgacgctggagagcttggccgccggcgtgcccgtcatcagctggcca

Nip    TTCTTCGCCGACCAGCAGACGAACTGCCGGTACCAGTGCAACGAGTGGGGCGTCGGCATGGAGATCGACAGCAACGTCAAGCGCGGCGCCGTCGCGTGCCTCATCGCTGAGCTCATGAA    1320
qRA2   TTCTTCGCCGACCAGCAGACGAACTGCCGGTACCAGTGCAACGAGTGGGGCGTCGGCATGGAGATCGACAGCAACGTCAAGCGCGGCGCCGTCGCGGGCCTCATCGCTGAGCTCATGAA    1320
qra2   TTCTTCGCCGACCAGCAGACGAACTGCCGGTACCAGTGCAACGAGTGGGGCGTCGGCATGGAGATCGACAGCAACGTCAAGCGCGGCGCCGTCGCGGGCCTCATCGCTGAGCTCATGGAA    1320
Consensttcttcgccgaccagcagacgaactgccggtaccagtgcaacgagtggggcgtcggcatggagatcgacagcaacgtcaagcgcggcgccgtcgcg gcctcatcgctgagctcatggaa

Nip    GGTCAGAAGGGGAAGGAGATGAGGAGGAAGGCGGAGGAGTGGAGGGAGAAGGCGATCAGGGCGGCTAAGCCCGGCGGCTCGTCTCACCGCAACTTCGAAGAGCTGGTCCGCCACGTGCTC    1440
qRA2   GGTCAGAAGGGGAAGGAGATGAGGAGGAAGGCGGAGGAGTGGAGGGAGAAGGCGATCAGGGCGGCTAAGCCCGGCGGCTCGTCTCACCGCAACTTCGAAGAGCTGGTCCGCCACGTGCTC    1440
qra2   GGTCAGAAGGGGAAGGAGATGAGGAGGAAGGCGGAGGAGTGGAGGGAGAAGGCGATCAGGGCGGCTAAGCCCGGCGGCTCGTCTCACCGCAACTTCGAAGAGCTGGTCCGCCACGTGCTC    1440
Consensggtcagaaggggaaggagatgaggaggaaggcggaggagtggagggagaaggcgatcagggcggctaagcccggcggctcgtctcaccgcaacttcgaagagctggtccgccacgtgctc

Nip    CTGGCCAAAGAGTCGTA    1457
qRA2   CTGGCCAAAGAGTCGTA    1457
qra2   CTGGCCAAAGAGTCGTA    1457
Consensctggccaaagagtcgta
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Figure S2. Multiple alignments of nucleic acid sequences. The nucleic acid sequences of Nip, *qRA2* and *qra2* in the LOC_Os02g51930 locus are displayed.

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Nip        MGSLGAAGKPHAVCMPYPAQGHITPMLNVAKLLHARGFDVTFVNTEYNHARLVRTRGEAAVAGAPGFRFATIPDGLPPSDDDVTQDIPSLCRSTKETCLAPFRRLLAQLNDPATGHPPV    120
qRA2       MGSLGAAGKPHAVCMPYPAQGHITPMLNVAKLLHARGFDVTFVNTEYNHARLVRTRGEAAVAGAPGFRFATIPDGLPPSDDDVTQDIPSLCRSTKETCLAPFRRLLAQLNDPATGHPPV    120
qra2       MGSLGAAGKPHAVCMPYPAQGHITPMLNVAKLLHARGFDVTFVNTEYNHARLVRTRGEAAVAGAPGFRFATIPDGLPPSDDDVTQDIPSLCRSTKETCLAPFRRLLAQLNDPATGHPPV    120
Consens    mgslgaagkphavcmpypaqghitpmlnvakllhargfdvtfvnteynharlvrtrgeaavagapgfrfatipdglppsddddvtqdipslcrstketclapfrrllaqlndpatghppv

Nip        TCVVSDVVMGFSMAAAKELGLPYVQLWTASSISYLGYRHYRLLMERGLAPLKDVDQLTNGYLDTPVEDVPGLRNMRIKDFPSFIHTTNPEEYMVGYVIEETERCKDASAIIVNSFGDLEG    240
qRA2       TCVVSDVVMGFSMAAAKELGLPYVQLWTASSISYLGYRHYRLLMERGLAPLKDVDQLTNGYLDTPVEDVPGLRNMRIKDFPSFIHTTNPEEYMVGYVIEETERCKDASAIIVNSFGDLEG    240
qra2       TCVVSDVVMGFSMAAAKELGLPYVQLWTASSISYLGYRHYRLLMR.GLAPLKDVDQLTNGYLDTPVEDVPGLRNMRIKDFPSFIHTTNPEEYMVGYVIEETERCKDASAIIVNSFGDLEG    239
Consens    tcvvsdvvmgfsmaaakelglpyvqlwtassisylgyrhyrllm glaplkdvdqltngyldtpvedvpglrnmrikdfpsfihttnpeeymvgyvieeterckdasaiivnsfgdleg

Nip        EAVAAMEALGLPKVYTLGPLPLVARKDPPSPRRSSIRLSLWKEQEECLQWLDGKEAGSVVYVNFGSITVMTNEQLVEFAWGLANSGREFLWIVRRDLVKGDTAVLPPEFLAETAERGLMA    360
qRA2       EAVAAMEALGLPKVYTLGPLPLVARKDPPSPRRSSIRLSLWKEQEECLQWLDGKEAGSVVYVNFGSITVMTNEQLVEFAWGLANSGREFLWIVRRDLVKGDTAVLPPEFLAETAERGLMA    360
qra2       EAVAAMEALGLPKVYTLGPLPLVARKDPPSPRRSSIRLSLWKEQEECLQWLDGKEAGSVVYVNFGSITVMTNEQLVEFAWGLANSGREFLWIVRRDLVKGDTAVLPPEFLAETAERGLMA    359
Consens    eavaamealglpkvytlgplplvarkdppsprrssirlslwkeqeeclqwldgkeagsvvyvnfgsitvmtneqlvefawglansgreflwivrrdlvkgdtavlppeflaetaerglma

Nip        SWCPQQDVLNHPAVGAFLTHSGWNSTLESLAAGVPVISWPFFADQQTNCRYQCNEWGVGMEIDSNVKRGAVACLIAELMEGQKGKEMRRKAEEWREKAIRAAKPGGSSHRNFEELVRHVL    480
qRA2       SWCPQQDVLNHPAVGAFLTHSGWNSTLESLAAGVPVISWPFFADQQTNCRYQCNEWGVGMEIDSNVKRGAVAGLIAELMEGQKGKEMRRKAEEWREKAIRAAKPGGSSHRNFEELVRHVL    480
qra2       SWCPQQDVLNHPAVGAFLTHSGWNSTLESLAAGVPVISWPFFADQQTNCRYQCNEWGVGMEIDSNVKRGAVAGLIAELMEGQKGKEMRRKAEEWREKAIRAAKPGGSSHRNFEELVRHVL    479
Consens    swcpqqdvlnhpavgaflthsgwnstleslaagvpviswpffadqqtncryqcnewgvgmeidsnvkrgava liaelmegqkgkemrrkaeewrekairaakpggsshrnfeelvrhvl

Nip        LAKE    484
qRA2       LAKE    484
qra2       LAKE    483
Consens    lake
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Figure S3. Multiple alignments of amino acid sequences. Amino acid sequences of Nip, *qRA2* and *qra2* in the LOC_Os02g51930 locus are displayed.