SUPPLEMENTAL INFORMATION

Base-Editing-Mediated Artificial Evolution of OsALS1 in planta to Develop Novel Herbicide-Tolerant Rice Germplasms

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Supplemental Figure 1. Gene Expression of *OsALS1*, *OsALS2* and *OsALS3*.
(A) Boxplot showing gene expression level of *OsALS1*, *OsALS2* and *OsALS3* in 9 tissues/284 experiments from Rice Expression Database (http://expression.ic4r.org). (B) RT-PCR Analysis of *OsALS1*, *OsALS2* and *OsALS3* in Kitaake. Genomic DNA was used as a control for sample quality and this experiment was repeated three times with the same results.
Supplemental Figure 2. The Genomic DNA Sequence and sgRNA-Targeting Sites of OsALS1 in Kitaake.

ATGGCTACGACCGCCGCGCCGCGCCGCGCCGACCTTGTCCGCCGCCGACGGCCAAGACCGGCCGTAAGAACCACCAGCGACACCACGTCCTTCCCGCTCCAGGCCGGGGTGTTGGGGACGCCTTGCCACCTCGCCCGGCAAGCCCAGGGCCGTGGGGCCGGCCGAGCCCCGCAAGGGCGCGGACATCCTCGTGGAGGCGCTGGAGCGGTGCGGCGTCAGCGACGTGTTCGCCTACCCGGGCGGCACGTCCATGGAGATCCACCAGGCGCTGACGCGCTCCCCGGTCATCACCAACCACCTCTCCGCCACGAGCAGGGCGAGGCGTTCGCGGCGTCCGGGTACGCGCGCGCGTCCGGCGCGTCGGGGTCTGCGTCGCCACCTCCGGCCCCGGGGCAACCACTCGTGTCCGCGCTCGCCGACGCGCTGCTCGACTCCGTCCCGATGGTCGCCATCACGGGCCAGGTCCCCCGCCGCATGATCGGCACCGACGCCTTCCAGGAGACGCCCATAGTCGAGGTCACCCGCATCCATCACCAAGCACAATTACCTTGTCCTTGATGTGGAGGACATCCCCCGCGTCATAAGGAAGCCTTCTTCCTCGCGTCCTCGGGCCGTCCTGGCCCGGTGCTGGTCGACATCCCAAGGACATCCAGCAGCAGATGGCTGTGCCAGTCTGGGACACCTCGATGAATCTACGGGGTACATTGCACGCCTGCCCAAGCCACCCGCGACAGAATTGCTTGAGCAGGTTTGGCGTCTGGTTGGCGAGTCACGGCGCCCGATTCTCTATGCGCCGGTGTGGTCTCTCTGACATCTGTGTAATTCCGCAACCAGCGAGATGTTAAGCTTGCTTTACAGGGCTTGAATGCTCTGCTAGACCAGAGCACAACAAAGACAAGTGTGATTGCTTGTGACAGGGAAAATTGAGGCTTTTGTACGCCAGTACATTCCACCCCTGGTACGCGCGCGCGCAGCAGCAGCGGTTTTTTTGATCAGCGCTGCCGCGGCGCGCAGTGGCTGTCTTCGGCTGGTCTGGGCGCAATGGGATTTGGGCTGCCTGCTGCAGCTGGTGCTTCTGTGGCTAACCCAGGTGTCACAGTTGTTGATATTGATGGGGATGGTACCTTCATGAACATTCAGGAGTTGGCATTGATCCGCATTGAGAACCTCCCGGTGAGGTGATGGTGTTGAACACCAACATTTGGGTATGGTTGTGCAATGGGAGGATAGTTTTACAAGGCAAATAGGGCGCATACATACTTGGGCAACCCAGAATGTGAGAGCGAGATATATCCAGATTTTGTGACTATTGCTAAAGGGTTCAATATTCCTGCAGTCCGTGTAACAAAGAAGAGTGAAGTCCGTGCCGCCATCAAGAAGATGCTCGAGACCCCAGGGCTACTTGTTGGATATCATCGTCCCACACCAGGAGCATGTGCTGCCTATGATCCCAAGTGAGGGGCGCATTCAAGGACATGATCCTGGATGGTGATGGCAGGACTATGTATTAA
Supplemental Figure 3. Nucleotide Changes of the Endogenous OsALS1 Gene in Rice Cells Introduced by BEMGE.

(A) Graphs of transition and transversion mutations in the target region of OsALS1 (261-666 bp) corresponding to sgRNA pool No. 2. sgRNA-binding regions are shown in different color, and sgRNA-unbinding regions are in black. Normalized reads were calculated as reads per million. (B) Summary of sgRNA numbers in transgenic callus lines introduced by Agrobacterium- and particle bombardment-mediated transformation. A total of 100 independent transgenic rice calli line were examined by Sanger sequencing. (C-E) Representative Sanger sequencing chromatograms of detected sgRNAs and nucleotide changes of OsALS1 in independent rice callus line #3 (C), line #16 (D) and line #13 (E) generated by Agrobacterium-mediated transformation. (F-G) Representative Sanger sequencing chromatograms of detected nucleotide changes of OsALS1 in independent rice callus line #7 (F) and line #34 (G) generated by particle bombardment-mediated transformation. In (C-G), the sgRNA transgenes and the nucleotide changes in target region of OsALS1 are underlined in the sequencing chromatograms.
Supplemental Figure 4. Evolved OsALS1 variant carrying point mutations at A152/A154/P171 sites identified in BS-tolerant plants from particle bombardment-mediated transformation.

The PAM sequences, the target sequences, the candidate bases in the putative editing window and the detected nucleotide changes/the corresponding amino acids are highlighted in green, bold, red and blue, respectively. The nucleotide changes are underlined in the sequencing chromatograms.
Supplemental Figure 5. BS-Tolerance Assay of wild-type Kitaake Seeds.
Wild-type Kitaake seeds were germinated in 1/2 MS cylinders complemented with 0, 0.1, 0.4, 0.6, 1 and 10 μM of BS, samples were photographed 14 days after BS treatment.
Supplemental Figure 6. Inheritance and BS-Tolerance Assay of OsALS1-Edited Plants.
(A) Germination assay of OsALS1-edited seeds in 1/2 MS cylinders with 0.6 μM of BS. Homozygous T2 seeds of P171L and P171S were used and the emergence of hypocotyl and roots was considered as tolerance. (B) The genotype of T0 line #9 and representatives of its T1 offspring. (C) Identification of transgene-free OsALS1(R190H) plants in T1 population. The presence and absence of individual genes were detected by PCR-amplification with gene-specific primers.
Supplemental Figure 7. Potential off-targets of rBE9/sgRNA19 in the rice genome.
The PAM sequences and the mismatches to the target sequence in the potential off-target region
are highlighted in green and red, respectively. The frequency for each off-target site is presented in
the columns to the right.
### Supplemental Table 1. List of Oligonucleotides in this Study.

| Primer name (5’ - 3’) | Primer name | Primer sequence (5’ - 3’) | Used for |
|-----------------------|-------------|---------------------------|----------|
| ccdB-F1               |             | ACCCGCGCGCTGTCGCTTGTGAGAGACCATTGCCGGCCATGAGG | inserting the ccdB-expression cassette into pENTR4:sgRNA8 |
| ccdB-R1               |             | CTTGCTATTTTCTAGCTCAAACTGAGACCCTGGCACCTGCAGACTGGC | |
| Array-F1              |             | CACATGCCCAGCTAATCCGAGCAACTTATAAACCCGCCC GTGTGTCGCTTGTGTCGGC | PCR-amplifying each sgRNA pool |
| Array-R1              |             | ACTCGGTGCTTTTCTAGTTGACAGTTGACTAGCTCTATTTAAACCTTGCTATTTCTAGCTTGAAG | |
| OsALS1-F1             |             | CCACACGATCCCGGAGTGA | detecting nucleotide changes of OsALS1 gene |
| OsALS1-R1             |             | TACCATGCAAGCAGCATCAAAC | |
| Oligo1                |             | CCCCGCGCGCTGCTGCTTGTGCTTAGCCGCCGCGGGCCTTTTAAGAGCTAGAAATAGCAAGTTAAATAAG | construction of sgRNA pool No.1 |
| Oligo2                |             | CCCCGCGCGCTGCTGCTTGTGCTTAGCCGCCGCGGGCCTTTTAAGAGCTAGAAATAGCAAGTTAAATAAG | |
| Oligo3                |             | CCCCGCGCGCTGCTGCTTGTGCTTAGCCGCCGCGGGCCTTTTAAGAGCTAGAAATAGCAAGTTAAATAAG | |
| Oligo4                |             | CCCCGCGCGCTGCTGCTTGTGCTTAGCCGCCGCGGGCCTTTTAAGAGCTAGAAATAGCAAGTTAAATAAG | |
| Oligo5                |             | CCCCGCGCGCTGCTGCTTGTGCTTAGCCGCCGCGGGCCTTTTAAGAGCTAGAAATAGCAAGTTAAATAAG | |
| Oligo6                |             | CCCCGCGCGCTGCTGCTTGTGCTTAGCCGCCGCGGGCCTTTTAAGAGCTAGAAATAGCAAGTTAAATAAG | |
| Oligo   | Sequence                                                                 | construction of sgRNA pool No.2 |
|---------|--------------------------------------------------------------------------|---------------------------------|
| Oligo7  | CCCGCGCGCTGTCGCTTTGTTGGCCGCGCTCCCCGCGGCGTTTTAGAGCTAGAAATAGCAAGTTAAAAATAAG |                                 |
| Oligo8  | CCCGCGCGCTGTCGCTTTGTTGGCCGCGCTCCCCGCGTTTTAGAGCTAGAAATAGCAAGTTAAAAATAAG  |                                 |
| Oligo9  | CCCGCGCGCTGTCGCTTTGTTGGCCGCGCTCCCCGCGTTTTAGAGCTAGAAATAGCAAGTTAAAAATAAG  |                                 |
| Oligo10 | CCCGCGCGCTGTCGCTTTGTTGGCCGCGCTCCCCGCGTTTTAGAGCTAGAAATAGCAAGTTAAAAATAAG  |                                 |
| Oligo11 | CCCGCGCGCTGTCGCTTTGTTGGCCGCGCTCCCCGCGTTTTAGAGCTAGAAATAGCAAGTTAAAAATAAG  |                                 |
| Oligo12 | CCCGCGCGCTGTCGCTTTGTTGGCCGCGCTCCCCGCGTTTTAGAGCTAGAAATAGCAAGTTAAAAATAAG  |                                 |
| Oligo13 | CCCGCGCGCTGTCGCTTTGTTGGCCGCGCTCCCCGCGTTTTAGAGCTAGAAATAGCAAGTTAAAAATAAG  |                                 |
| Oligo14 | CCCGCGCGCTGTCGCTTTGTTGGCCGCGCTCCCCGCGTTTTAGAGCTAGAAATAGCAAGTTAAAAATAAG  |                                 |
| Oligo15 | CCCGCGCGCTGTCGCTTTGTTGGCCGCGCTCCCCGCGTTTTAGAGCTAGAAATAGCAAGTTAAAAATAAG  |                                 |
| Oligo16 | CCCGCGCGCTGTCGCTTTGTTGGCCGCGCTCCCCGCGTTTTAGAGCTAGAAATAGCAAGTTAAAAATAAG  |                                 |
| Oligo17 | CCCGCGCGCTGTCGCTTTGTTGGCCGCGCTCCCCGCGTTTTAGAGCTAGAAATAGCAAGTTAAAAATAAG  |                                 |
| Oligo18 | CCCGCGCGCTGTCGCTTTGTTGGCCGCGCTCCCCGCGTTTTAGAGCTAGAAATAGCAAGTTAAAAATAAG  |                                 |
| Oligo19 | CCCGCGCGCTGTCGCTTTGTTGGCCGCGCTCCCCGCGAGTTTTAGAGCTAGAAATAGCAAGTTAAAAATAAG |                                 |
| Oligo   | Sequence                                                                 | construction of sgRNA pool No.3 |
|---------|--------------------------------------------------------------------------|----------------------------------|
| Oligo20 | CCCGCAGCTGGATCGGCAACCCGACCTCCCGGTTTT AGAGCTAGGAATAGCAAGTTAAAATAAG        |                                  |
| Oligo21 | CCCGCAGCTGGATCGGCAACCCGACCTCCCGGTTTT AGAGCTAGGAATAGCAAGTTAAAATAAG        |                                  |
| Oligo22 | CCCGCAGCTGGATCGGCAACCCGACCTCCCGGTTTT AGAGCTAGGAATAGCAAGTTAAAATAAG        |                                  |
| Oligo23 | CCCGCAGCTGGATCGGCAACCCGACCTCCCGGTTTT AGAGCTAGGAATAGCAAGTTAAAATAAG        |                                  |
| Oligo24 | CCCGCAGCTGGATCGGCAACCCGACCTCCCGGTTTT AGAGCTAGGAATAGCAAGTTAAAATAAG        |                                  |
| Oligo25 | CCCGCAGCTGGATCGGCAACCCGACCTCCCGGTTTT AGAGCTAGGAATAGCAAGTTAAAATAAG        |                                  |
| Oligo26 | CCCGCAGCTGGATCGGCAACCCGACCTCCCGGTTTT AGAGCTAGGAATAGCAAGTTAAAATAAG        |                                  |
| Oligo27 | CCCGCAGCTGGATCGGCAACCCGACCTCCCGGTTTT AGAGCTAGGAATAGCAAGTTAAAATAAG        |                                  |
| Oligo28 | CCCGCAGCTGGATCGGCAACCCGACCTCCCGGTTTT AGAGCTAGGAATAGCAAGTTAAAATAAG        |                                  |
| Oligo29 | CCCGCAGCTGGATCGGCAACCCGACCTCCCGGTTTT AGAGCTAGGAATAGCAAGTTAAAATAAG        |                                  |
| Oligo30 | CCCGCAGCTGGATCGGCAACCCGACCTCCCGGTTTT AGAGCTAGGAATAGCAAGTTAAAATAAG        |                                  |
| Oligo31 | CCCGCAGCTGGATCGGCAACCCGACCTCCCGGTTTT AGAGCTAGGAATAGCAAGTTAAAATAAG        |                                  |
| Oligo32 | CCCGCAGCTGGATCGGCAACCCGACCTCCCGGTTTT AGAGCTAGGAATAGCAAGTTAAAATAAG        |                                  |
| Oligo33  | CCCGCCTGCGCTTGTGCGAGATCTGTGAGGATTTTAGAGCTAGAAATGCAAGTCAGTTAAATAAG |
| Oligo34  | CCCGCGCGCTGCGCTTGTGCGAGATCTGTGAGGATTTTAGAGCTAGAAATGCAAGTCAGTTAAATAAG |
| Oligo35  | CCCGCGCGCTGCGCTTGTGCGAGATCTGTGAGGATTTTAGAGCTAGAAATGCAAGTCAGTTAAATAAG |
| Oligo36  | CCCGCGCGCTGCGCTTGTGCGAGATCTGTGAGGATTTTAGAGCTAGAAATGCAAGTCAGTTAAATAAG |
| Oligo37  | CCCGCGCGCTGCGCTTGTGCGAGATCTGTGAGGATTTTAGAGCTAGAAATGCAAGTCAGTTAAATAAG |
| Oligo38  | CCCGCGCGCTGCGCTTGTGCGAGATCTGTGAGGATTTTAGAGCTAGAAATGCAAGTCAGTTAAATAAG |
| Oligo39  | CCCGCGCGCTGCGCTTGTGCGAGATCTGTGAGGATTTTAGAGCTAGAAATGCAAGTCAGTTAAATAAG |
| Oligo40  | CCCGCGCGCTGCGCTTGTGCGAGATCTGTGAGGATTTTAGAGCTAGAAATGCAAGTCAGTTAAATAAG |
| Oligo41  | CCCGCGCGCTGCGCTTGTGCGAGATCTGTGAGGATTTTAGAGCTAGAAATGCAAGTCAGTTAAATAAG |
| Oligo42  | CCCGCGCGCTGCGCTTGTGCGAGATCTGTGAGGATTTTAGAGCTAGAAATGCAAGTCAGTTAAATAAG |
| Oligo43  | CCCGCGCGCTGCGCTTGTGCGAGATCTGTGAGGATTTTAGAGCTAGAAATGCAAGTCAGTTAAATAAG |
| Oligo44  | CCCGCGCGCTGCGCTTGTGCGAGATCTGTGAGGATTTTAGAGCTAGAAATGCAAGTCAGTTAAATAAG |
| Oligo45  | CCCGCGCGCTGCGCTTGTGCGAGATCTGTGAGGATTTTAGAGCTAGAAATGCAAGTCAGTTAAATAAG |
| Oligo   | Sequence                                                                 | Notes                                      |
|--------|--------------------------------------------------------------------------|--------------------------------------------|
| Oligo46| `CCCGCGCGCTGCTGCTTTGTGCTGCTGACGTGGTCTTCGTTCTGGTTTTA GAGCTAGAAATAGCAAGTTAAAAATAAG` |                                            |
| Oligo47| `CCCGCGCGCTGCTGCTTTGTGCTGCTGACGTGGTCTTCGATTAGTTAATTGAGTTTT AGAGCTAGAAATAGCAAGTTAAAAATAAG` |                                            |
| Oligo48| `CCCGCGCGCTGCTGCTTTGTGCTGCTGACGTGGTCTTCGATTAGTTAATTGAGTTTT AGAGCTAGAAATAGCAAGTTAAAAATAAG` |                                            |
| Oligo49| `CCCGCGCGCTGCTGCTTTGTGCTGCTGACGTGGTCTTCGATTAGTTAATTGAGTTTT AGAGCTAGAAATAGCAAGTTAAAAATAAG` |                                            |
| Oligo50| `CCCGCGCGCTGCTGCTTTGTGCTGCTGACGTGGTCTTCGATTAGTTAATTGAGTTTT AGAGCTAGAAATAGCAAGTTAAAAATAAG` |                                            |
| Oligo51| `CCCGCGCGCTGCTGCTTTGTGCTGCTGACGTGGTCTTCGATTAGTTAATTGAGTTTT AGAGCTAGAAATAGCAAGTTAAAAATAAG` |                                            |
| Oligo52| `CCCGCGCGCTGCTGCTTTGTGCTGCTGACGTGGTCTTCGATTAGTTAATTGAGTTTT AGAGCTAGAAATAGCAAGTTAAAAATAAG` |                                            |
| Oligo53| `CCCGCGCGCTGCTGCTTTGTGCTGCTGACGTGGTCTTCGATTAGTTAATTGAGTTTT AGAGCTAGAAATAGCAAGTTAAAAATAAG` |                                            |
| Oligo54| `CCCGCGCGCTGCTGCTTTGTGCTGCTGACGTGGTCTTCGATTAGTTAATTGAGTTTT AGAGCTAGAAATAGCAAGTTAAAAATAAG` |                                            |
| Oligo55| `CCCGCGCGCTGCTGCTTTGTGCTGCTGACGTGGTCTTCGATTAGTTAATTGAGTTTT AGAGCTAGAAATAGCAAGTTAAAAATAAG` |                                            |
| Oligo56| `CCCGCGCGCTGCTGCTTTGTGCTGCTGACGTGGTCTTCGATTAGTTAATTGAGTTTT AGAGCTAGAAATAGCAAGTTAAAAATAAG` |                                            |
| Oligo57| `CCCGCGCGCTGCTGCTTTGTGCTGCTGACGTGGTCTTCGATTAGTTAATTGAGTTTT AGAGCTAGAAATAGCAAGTTAAAAATAAG` |                                            |
| Oligo58| `CCCGCGCGCTGCTGCTTTGTGCTGCTGACGTGGTCTTCGATTAGTTAATTGAGTTTT AGAGCTAGAAATAGCAAGTTAAAAATAAG` |                                            |

construction of sgRNA pool No.6
| Oligo59   | CCCGCGCGCTGCTGGCTTGTATCCAAACAGTATGGCCCGTTTTAGAGCTAGAAATAGCAAGTTAAAATAAG |
| Oligo60   | CCCGCGCGCTGCTGGCTTGTATGGCTCTGCTGGTTTTAAGAGCTAGAAATAGCAAGTTAAAATAAG |
| Oligo61   | CCCGCGCGCTGCTGGCTTGTATGGCTGCTGGTTTTAAGAGCTAGAAATAGCAAGTTAAAATAAG |
| Oligo62   | CCCGCGCGCTGCTGGCTTGTATGGCTGCTGGTTTTAAGAGCTAGAAATAGCAAGTTAAAATAAG |
| Oligo63   | CCCGCGCGCTGCTGGCTTGTATGGCTGCTGGTTTTAAGAGCTAGAAATAGCAAGTTAAAATAAG |
| OsALS2-F1 | CAAGGACATCCAGCAGCAGA          | semi-quantitative RT-PCR analysis of OsALS2 |
| OsALS2-R1 | GAAGTTCCCGATGCCCATGA          |
| OsALS3-F1 | GATGCATGGCAGCTGTGTACG          | semi-quantitative RT-PCR analysis of OsALS3 |
| OsALS3-R1 | TTGCCTGGTGTGGTTCCAG          |
| OsActin-F1 | GCGTGAGACAAAATTTCTTCAACCG      | semi-quantitative RT-PCR analysis as the internal control |
| OsActin-R1 | TCTGTACCCCTCATCAGGCATC |
| Hyg-F1    | CGGAAGTGCTTGACATTG          | PCR-amplifying Hyg gene in OsALS1-editing plants |
| Hyg-R1    | GACCTCGATATTGGAATCC          |
| Cas9-F1   | GGTAATGAACACTCGCTCTGC          | PCR-amplifying Cas9 gene in OsALS1-editing plants |
| Cas9-R1   | TGCCGTCAAGAATCTCCTTTG          |
| U6p-F3    | CTGTGATGGGCTGGATG          | PCR-amplifying sgRNA cassette in |

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| Sequence 1   | Sequence 2   | Description                                                                 |
|-------------|-------------|-----------------------------------------------------------------------------|
| NosT-g10-R1 | CGTTATTTATGAGATGGGTTT | *OsALS1*-editing plants                                                     |
| U6p-OsALS1-F1 | GAGGCAGGGAGGAACAGTTTAG | Deep-sequencing of sgRNAs in transgenic calli obtained by *A. tumefaciens*-mediated transformation |
| NosT-OsALS1-R1 | CGCCAGTGTGATGGATATCTG | Deep-sequencing of sgRNAs in transgenic calli obtained by gene gun bombardment |
| U6p-OsALS1-F2 | GAGAGGCGGGAGGAACAGT | Deep-sequencing of sgRNAs in transgenic calli obtained by gene gun bombardment |
| NosT-OsALS1-R2 | TCTGCAGAAATTGCCCCTTCG | Deep-sequencing of the target region of *OsALS1* in transgenic calli obtained by *A. tumefaciens*-mediated transformation |
| OsALS1-DS-F1 | CGACGTGTTCTCGCCTACC | Deep-sequencing of the target region of *OsALS1* in transgenic calli obtained by *A. tumefaciens*-mediated transformation |
| OsALS1-DS-R1 | GCCATCTGCTGCTGGAT | Deep-sequencing of the target region of *OsALS1* in transgenic calli obtained by *A. tumefaciens*-mediated transformation |
| OsALS1-DS-F2 | TGGAGCGGTGCGGCCGTCA | Deep-sequencing of the target region of *OsALS1* in transgenic calli obtained by *A. tumefaciens*-mediated transformation |
| OsALS1-DS-R2 | GCCAGGACGGCCCGAGGAC | Deep-sequencing of the target region of *OsALS1* in transgenic calli obtained by *A. tumefaciens*-mediated transformation |
| OsALS1-off1-F1 | GAGCCGCTACAAGCGAGGT | Off-target detection of the target site sgRNA19 |
| OsALS1-off1-R1 | CGGGAGATGTATCCCGCA | Off-target detection of the target site sgRNA19 |
| OsALS1-off2-F1 | CGCCGCTAAGCTGCCCCAACCG | Off-target detection of the target site sgRNA19 |
| OsALS1-off2-R1 | CGACCCGTTGGACGCGGAGGAAGAA | Off-target detection of the target site sgRNA19 |
METHODS

Rice Cultivars and Growth Conditions

The *Geng* rice cultivar Kitaake and Nangeng 46 were used in this study. Both rice varieties were kept in our lab. Wild-type and *OsALS1*-edited plants were grown in a growth chamber under short-day conditions (10 h light at 30°C / 14 h dark at 25°C) or in the greenhouse under natural conditions in Beijing.

*Agrobacterium tumefaciens*-Mediated Rice Transformation

*Agrobacterium*-mediated rice transformation was carried out with immature embryo of rice seed following a protocol mentioned previously (Hiei and Komari, 2008) with minor modifications. Briefly, each rBEs/sgRNAs pool was individually transferred into *A. tumefaciens* strain EHA105 by electroporation, all *Agrobacterium* cells were scraped off the plates, collected in MSD liquid medium, adjusted the final OD_{600} to 0.1, and then used to infect 14-day-old rice calli. After two rounds of Hygromycin (Roche) selection on MSD plates, the resistant calli were screened for herbicide resistance with 0.4 μM Bispyribac-sodium (Sigma) on MSD plates in the next 4 weeks. Rice plants were further generated from independent herbicide-resistant callus lines.

Particle Bombardment-Mediated Rice Transformation

Gold particle preparation and plasmid DNA delivery was carried out following a protocol (Christou, 1997) with minor modifications. Briefly, 0.75 mg of gold particle was coated with 4 μg of plasmids of each rBEs/sgRNAs pool and used for 4 shots of particle bombardments by the Biolistic PDS-1000/He Particle Delivery System (Bio-Rad). Each plate of rice calli was first bombarded with a 1,100 psi rupture disk at 10-cm distance down from the stopping screen, and it was repeated with a 650 psi rupture disk at 6-cm distance. After two rounds of Hygromycin (Roche) selection on MSD plates, the resistant calli were screened for herbicide resistance with 0.4 μM Bispyribac-sodium (Sigma) on MSD plates in the next 4 weeks. Rice plants were generated from independent herbicide-resistant callus lines finally.
RNA Extraction and RT-PCR Analysis
Total RNA was extracted from leaves of each independent rice line with the TRIzol™ Reagent (Invitrogen) and 1 μg of RNA from each sample was used as template to synthesize the first-strand cDNA using the PrimeScript™ RT Reagent Kit with gDNA Eraser (Takara Bio) following the manufacturer’s instructions. RT-PCR was carried out with gene specific primers (Supplemental Table 1) with varying numbers of amplification cycles (25, 30, 35) and OsActin as an internal control. These experiments were repeated three times.

Genomic DNA Extraction and Genotyping
Genomic DNA was extracted from calli and rice leaves using Plant Genomic DNA Kit (TIANGEN) as described by the manufacturer, and used as the template to PCR amplify the corresponding target region of OsALS1, the transgenes Cas9, Hyg, sgRNA or OsALS1 mutant alleles with I5 high-fidelity DNA polymerase (MCLAB) and specific primers (Supplemental Table 1). Amplicons were gel-purified and subjected to Sanger sequencing directly. For low quality of sequencing results, the PCR products were further cloned into the pEASY-Blunt cloning vector (TransGen Biotech), and a number of colonies identified with inserts were sequenced.

Herbicide Tolerance Assay
Rice calli of wild-type Kitaake were cultured on MSD plates containing a concentration gradient of bispyribac-sodium (Sigma) at 0, 0.1, 0.25, 0.4, 0.52 and 1 μM, respectively, phenotypes were then recorded after 28 days. Rice seeds were germinated in 1/2 MS cylinders containing a concentration gradient of bispyribac-sodium (Sigma) at 0, 0.1, 0.4, 0.6, 1 and 10 μM, respectively, phenotypes were then recorded after 14 days. Three-week-old rice seedlings were sprayed with bispyribac-sodium suspension concentrate (Mefront) at 16x, 32x, 48x (x = 26.25 g a.i./ha) using spray tower at 0.3 MPa pressure, phenotypes were then recorded after 21-30 days of herbicide applications, wild-type plants were included as the control. These experiments were repeated three times.
REFERENCES

Christou, P. (1997). Rice transformation: bombardment. Plant Mol. Biol. 35:197-203.

Hiei, Y., and Komari, T. (2008). *Agrobacterium*-mediated transformation of rice using immature embryos or calli induced from mature seed. Nat. Protoc. 3:824-834.