### Supplemental Information

**Improving adenine base editing efficiency in rice by simplifying base editors**

| Contents |
|-----------------------------|
| **Supplemental sequence** The DNA sequences of key components in the different adenine base editors |
| **Supplemental Figure 1** Off-target editing at *OsSPL17* by ABE-P1S. |
| **Supplemental Table 1** The base editing windows of ABE-P1 and ABE-P1S at different target sites. |
| **Supplemental Table 2** The base editing windows of ABE-P2, ABE-P2S, ABE-P5 and ABE-P5S at different target sites. |
| **Supplemental Table 3** The base editing frequencies of ABE-P1 and ABE-P1S at potential off-target sites of sgRNA1. |
| **Supplemental Table 4** The base editing frequencies of ABE-P1 and ABE-P1S at potential off-target sites of sgRNA6. |
| **Supplemental Table 5** The base editing frequencies of ABE-P2S at potential off-target sites of sgRNA9. |
| **Supplemental Table 6** The target sites and primers for all sgRNAs used in this study. |
| **Supplemental Table 7** Primers for on-target site amplification and sequencing. |
| **Supplemental Table 8** Primers for potential off-target site amplification and sequencing. |
Supplemental sequence The DNA sequences of key components in the different adenine base editors.

Wild type ecTadA-32aa linker

ATGTCCGAAGTCGAGTTTTCCCCATGAGTAATTCGAGATGACACGCATTGACT
CTCGCAAGAGGAGCCGTTGGAAGTACGCGAGGTCGCGCCGTCGGGGGAGTCGCT
CGTGCATAAACATCGGTAATCGGCGAGGCTTTGGAAGTACGCGAGGTCGCT
CCACGACCCCAATCCGCAATACGCCAGGCTTCTGCGACAGGTCGCT
TGATATCGGTTCGCCGACGCGAGGTCGCGCCGTCGGGGGAGTCGCT
ACGTGCTGCACTACCCAGCAGTGAACCACCAGGCTTCTGCGACAGGTCGCT
ATATGGCGGAGCAAGTCGCGAGGTCGCGCCGTCGGGGGAGTCGCT
AGGCAAGCGGAGATGACAGGTCGCGCCGTCGGGGGAGTCGCT
ecTadA*7.10-32aa linker

ATGTCCGAAGTCGAGTTTTCCCCATGAGTAATTCGAGATGACACGCATTGACT
CTCGCAAGAGGAGCCGTTGGAAGTACGCGAGGTCGCGCCGTCGGGGGAGTCGCT
CGTGCTTAACAATCGGTAATCGGCGAGGCTTTGGAAGTACGCGAGGTCGCT
CCACGACCCCAATCCGCAATACGCCAGGCTTCTGCGACAGGTCGCT
TGATATCGGTTCGCCGACGCGAGGTCGCGCCGTCGGGGGAGTCGCT
ACGTGCTGCACTACCCAGCAGTGAACCACCAGGCTTCTGCGACAGGTCGCT
ATATGGCGGAGCAAGTCGCGAGGTCGCGCCGTCGGGGGAGTCGCT
AGGCAAGCGGAGATGACAGGTCGCGCCGTCGGGGGAGTCGCT
nSpCas9 (D10A)-NLS

GACAAGAAGTACAGCATCGGCAAGGCTGGCCCTGGCCATTGAGAGAAGACGCATTGACT
CTCGCAAGAGGAGCCGTTGGAAGTACGCGAGGTCGCGCCGTCGGGGGAGTCGCT
CGTGCTTAACAATCGGTAATCGGCGAGGCTTTGGAAGTACGCGAGGTCGCT
CCACGACCCCAATCCGCAATACGCCAGGCTTCTGCGACAGGTCGCT
TGATATCGGTTCGCCGACGCGAGGTCGCGCCGTCGGGGGAGTCGCT
ACGTGCTGCACTACCCAGCAGTGAACCACCAGGCTTCTGCGACAGGTCGCT
ATATGGCGGAGCAAGTCGCGAGGTCGCGCCGTCGGGGGAGTCGCT
AGGCAAGCGGAGATGACAGGTCGCGCCGTCGGGGGAGTCGCT
GCTACGCCCCCCATCTCGAGGAGGTCGCTCGCGCCGTCGGGGGAGTCGCT
GCCTACGCCGACCCATCTCGAGGAGGTCGCTCGCGCCGTCGGGGGAGTCGCT
nSpCas9 (D10A)-NLS

GACAAGAAGTACAGCATCGGCAAGGCTGGCCCTGGCCATTGAGAGAAGACGCATTGACT
CTCGCAAGAGGAGCCGTTGGAAGTACGCGAGGTCGCGCCGTCGGGGGAGTCGCT
CGTGCTTAACAATCGGTAATCGGCGAGGCTTTGGAAGTACGCGAGGTCGCT
CCACGACCCCAATCCGCAATACGCCAGGCTTCTGCGACAGGTCGCT
TGATATCGGTTCGCCGACGCGAGGTCGCGCCGTCGGGGGAGTCGCT
ACGTGCTGCACTACCCAGCAGTGAACCACCAGGCTTCTGCGACAGGTCGCT
ATATGGCGGAGCAAGTCGCGAGGTCGCGCCGTCGGGGGAGTCGCT
AGGCAAGCGGAGATGACAGGTCGCGCCGTCGGGGGAGTCGCT
GCTACGCCCCCCATCTCGAGGAGGTCGCTCGCGCCGTCGGGGGAGTCGCT
GCCTACGCCGACCCATCTCGAGGAGGTCGCTCGCGCCGTCGGGGGAGTCGCT
nSpCas9 (D10A)-NLS

GACAAGAAGTACAGCATCGGCAAGGCTGGCCCTGGCCATTGAGAGAAGACGCATTGACT
CTCGCAAGAGGAGCCGTTGGAAGTACGCGAGGTCGCGCCGTCGGGGGAGTCGCT
CGTGCTTAACAATCGGTAATCGGCGAGGCTTTGGAAGTACGCGAGGTCGCT
CCACGACCCCAATCCGCAATACGCCAGGCTTCTGCGACAGGTCGCT
TGATATCGGTTCGCCGACGCGAGGTCGCGCCGTCGGGGGAGTCGCT
ACGTGCTGCACTACCCAGCAGTGAACCACCAGGCTTCTGCGACAGGTCGCT
ATATGGCGGAGCAAGTCGCGAGGTCGCGCCGTCGGGGGAGTCGCT
AGGCAAGCGGAGATGACAGGTCGCGCCGTCGGGGGAGTCGCT
GCTACGCCCCCCATCTCGAGGAGGTCGCTCGCGCCGTCGGGGGAGTCGCT
GCCTACGCCGACCCATCTCGAGGAGGTCGCTCGCGCCGTCGGGGGAGTCGCT
nSpCas9 (D10A)-NLS

GACAAGAAGTACAGCATCGGCAAGGCTGGCCCTGGCCATTGAGAGAAGACGCATTGACT
CTCGCAAGAGGAGCCGTTGGAAGTACGCGAGGTCGCGCCGTCGGGGGAGTCGCT
CGTGCTTAACAATCGGTAATCGGCGAGGCTTTGGAAGTACGCGAGGTCGCT
CCACGACCCCAATCCGCAATACGCCAGGCTTCTGCGACAGGTCGCT
TGATATCGGTTCGCCGACGCGAGGTCGCGCCGTCGGGGGAGTCGCT
ACGTGCTGCACTACCCAGCAGTGAACCACCAGGCTTCTGCGACAGGTCGCT
ATATGGCGGAGCAAGTCGCGAGGTCGCGCCGTCGGGGGAGTCGCT
AGGCAAGCGGAGATGACAGGTCGCGCCGTCGGGGGAGTCGCT
GCTACGCCCCCCATCTCGAGGAGGTCGCTCGCGCCGTCGGGGGAGTCGCT
TCAAGACCGAGATTACCGCTGCGCAACGGCGAGATCCGGAAGCGGCCTCTGATCG

AGACAAACGCGCAGAACCCGACGCAGACTGCTGTGGAATAGGGCGCCGAGTTGTC

CCGTCGCGAAAAAGTGTCTGAGACATGCCCAAGGTGAAATCGTGAGAAAAAGACGAG

TCGACAGACGGGCCTTCAGCAAAGAGATCTATCTTCGTCCCAAGAGGAACAGCAGTA

AGCTGATCCAGAAGATCCAGATCGGACCCATTAGTCTCGTGAAAAAGACGAG

GCCCAACCGGTGCTCTTCTGCTGTGGTGGGACCAAAGTGGAAGGGCAAGT

CCAGAAGAACTGAGAGGTGGAAAGAACGCTCGCTGGGGCCAGTACGAGAAGGAGAAC

TGGCCCTGGCCCTCCCAATATCGTCTCTGACCTACTCCAGCCAGACTTAA

GCTGAAGGGGTCCGCCAGATGAGAAGACTGCTGTGTTTGAGGAACAGCA

CAAGCAGAATGTTAGATGAAACAGCTGCTGTTTACCTGAGAAAGT

nSacCas9 (D10A)-NLS

AAGCGGAAACTCATCTCTGGGCTGCGCATCAGACGAGTTGGCTACCGGC

ATCATGCAGAGATCGAGATGCTGAGTAGCCGGCTGCTGCTGTCTCAA

GAGGCCAACCTGGGAAAATACGAGAGGCAGAGCCGAGGGGAAGAGAGCCCAAGG

GCTGAAAGCGGAGACGGGCATACGCTACGAGACGGGACGTGATCGATGCCGGCGTGCGGCTGTTCAAA

AGGTTACAGCACAGCAACAAAGGGGATGCGTTGAGACGCCAAGCGCTGAGGACTAC

ACCAGCCTGACATCATAGACAGGCAAGGCAAGGAAGGCGCCAGTCAAAAGCCAGAC

CATGGAAGGGCCTGCAATGACGAGAGTGAAGAAGCTGCTGTTCGAA

CTACAACCTTGCAGTGAAGGAGCCAGAAGGCGTACGCTGCAAAGGAAAG

AAGCTGCAAGCTAGGTCTCCCAATCCGGCGGATTTCAGCGTACCCAGAAATG

GCTGACCTGGCCCAAGAGAAGAGGCGTGCACAACGTGAACGAGGTGGAAGAG

CAGACCGAGATGCTGAGATGTTCTGTGCTGACGTGGCAAAATTGGAGT

AAGCGCAAACGTGCAAGAGGTATA

GGATTACCATATTTAAAAGCGGCTTCTCCAAAGCGTCCGCGTGACCGTCACGATGGAGAATTGGGTG
TGCTGCACCTGGCCAAGAGAAGAGGCGTGCACAACGTGAACGAGGTGGAAGAG
203
GACACCGGCAACACGAGCTGTCAGACCAACAGAGCAGATCAGCCGGAACAGCAAGGC
204
CCTGGAAGAGAAAACTGCGGCAACTGCAGCTGGAACGGCTGAAGAAAGACG
205
GCGAAGTGCGGGGACGACATCAACAGATTTCAAGACAGCAGACTACGTGAAAGAG
206
CCCAACAGCTCAGTCAGACCTGGAAGGCCTACCAACAGCTGGAACAGCTGGAAGTCTCA
207
TGACACCTACATCGACCTGCTGGAAACCCGGCGGACCTACTATGAGGGACCTG
208
GCGAGGGCAGCCCCTTCGGCTGGAAGGACATCAAAGAATGGTACGAGATGCTGA
209
TGGGCCACTGCACCTACTTCCCCGAGGAACTGCGGAGCGTGAAG
210
ACGCCGACCTGTACAACGCCCTGAACGACCTGAACAATCTCGTGATCACCAGGG
211
ACGAGAACGAGAAGCTGGAATATTACGAGAAGTTCCAGATCATCGAGAACGTGTT
212
CAAGAAGAAGAAGAAGCCCACCCTGAAGCAGATCGCCAAAGAAATCCTCGTGAA
213
CGAAGAGGATATTAAGGGCTACAGAGTGACCAGCACCGGCAAGCCCGAGTTCAC
214
CAACCTGAAGGTGTACCACGACATCAAGGACATTACCGCCCGGAAAGAGATTATT
215
GAGAACGCCGAGCTGCTGGATCAGATTGCCAAGATCCTGACCATCTACCAGAGC
216
AGCGAGGACATCCAGGAAGAATCGCAACTCCTGAACTCCGAGCTGACCCAGGAA
217
GAGATCGAGCAGATCTCTAATCTGAAGGGCTATACCGGCACCCACAACCTGAGCC
218
TGAAGGCCATCAACCTGATCCTGGACGAGCTGTGGCACACCAACGACAACAGG
219
TCGCTATCTTCCAACGGCGTGAACGTCGTGGAACGGCTGCAAGGAAATCTCCATCGTCCAGC
220
AGAAAGAGATCCCCACCACCCTGGTGGACGACTTCATCCTGAGCCCCGTCGTGA
221
AGAGAAGGCTTATCCAGAGCATCAAAGTGATCAACGCCCATCATCAAGAAGTACGG
222
CCTGCCCAACGACATCATTATCGAGCTGGCCCGCGAGAAGAACTCCAAGGACGC
223
CCAGAAAATGATCAACAGGACGAAAGCGGGAACCCGCGAGCAACACGGGAGGAT
224
CGAGGAATCATCCCGGAACCACCGGCACCAAGAAGGCACAAGTACCTGACGAGA
225
GATCGACGTGCAAGACATGCAAGGCAAGGCAAAGTGCCTGTACAGCCTGGAAGCCAT
226
CCCTCTGGAAGATCTGCTGAACAACCCCTTCAACTATGAGGTGGACACATCATC
227
CCCAAGGGCTGTCTCTTGCACAAACAAGCTTCAACAAACAAAGTCTCGTGAAGACG
228
GAAGAAAACACGCAAGAAGAGCCAAGGGCAACCCCATCCATCGATCAGCGACGC
229
GACAGCAAGATCGCTACGAAAACCTTCAAGAAGGCAACATCTCGGAAATCTGGGCAAG
230
GGCAAGGCCAATGCAAGCAAGACAAAGAAAGATGACTCTGGAACAGAACGGGAC
231
ATCAACAGGTTTCTCCGTGCAGAAAGACTTCTATCAACCCGAACCTGTGTGATACCA
232
GATAGGCCACCCAGAGGCGCTGTGAAACCTGCTGCGGAGCTACTTCATCTAGGTGAACA
233
ACCTGGAAGTGCCACTCATCAATGCGGCGCTTCAACGGCTTCCTCTGCGGC
234
GGAACTGGAAGTTAAAGAAAGCGCAAAACAGGGTATCAGAAGCACAGCACCCCGG
235
GACCCTCATGTACTGCAAAACGGCCACAGATCTTTCATCAAAGGACCCCGCGAG
236
GACGCCCTGATCCAGCAACTAGCAAGATTACGAGAAGTTCCAGATCATCGAGAACGTGTT
237
ACAAGGCAAAAGTTGCTGGAAGAAGCCGCAAAGATGCGTTCGGAAGAAGATCTGG
238
GCTGATCAGACGTGAAACCTCCGCAGATCGAGAAGGCTGGAACGCTACGACCCGGAA
239
GACCAGAAGAAGTCCCGTTCGCAGATCAGACGTGAAACCTCCGCAGATCGAGAAGGCTGGAACGCTACGACCCGGAA
240
CGACAAAGGCAACCCCTGATCGTGAACAACTCTGGAACGCGCGCTTGACGACAAGGA
241
CAATGCAAGCTGAAAGCTGAAAGACTGATCATCAACAGGACAGCCGGAAAGGGCTGGAACGCTACGACCCGGAA
242
CCACACCAAGCCCCAGACCTACAGAAGAATCGGATATTAGGAAACGATCCACG
243
CGACAGAAGAAGTCCCGTTCGCAGATCAGACGTGAAACCTCCGCAGATCGAGAAGGCTGGAACGCTACGACCCGGAA
244
CAAGTCATCCAAAAAGGAAACACCGGCGCCCGTCTCATGCAAGATGATTAAAGTATGACG
245
AACAAAAGTTCAGACCCCATCTGGAACATCAGCGACACCTCACCACGCAACAGAAAC
AAGGTCGTGAAGCTGTCCCTGAAGCCCTACAGATTCGACGTGTACCTGGACAAT
GGCGTGTACAAGTTCGTGACCGTGAAGAATCTGGATGTGATCAAAAAAGAAAACT
ACTACGAAAGTGATAGCAAGTGCTATGAGGAAGCTAAGAAG
CTGAAGAAGATCA
GCAACCAGGCCAGTTTATCGCCTCCTCTCTCAAAAGAGATCTGATCAAGATCAA
CGGCGAGCTGTATAGATCGATCGCCTCAACATCTGAGAACAACCGACTTGCTGAAACCGGATCGA
AGTGAACATGATCGACATCAMCTACCACCAGGTACCTGAGAAAACATGAACGACAG
AGCCCCCCCCATCATCATTAAAGCAATCGCCTCATAAGACACAGACATTAAGAAGT
ACAGCACAGACATTCTGGGCAACCTGTATGAAGTGAAATCTAAGAAGCACCCTCA
GATCATCAAAAAGGGACTAGTTCCGCGCGGCAGCGCTTCTCCAAAGCGTCCCGCGTGAC
CGTCACGATGAAGATGTTGGTGAGGACGCAAAACGTGCAAAGGTGTTAA


**Supplemental Figure 1.** Off-target editing at *OsSPL17* by ABE-P1S. **a,** Schematic view of the potential off-target site 1 of sgRNA1 in *OsSPL17*. The mismatch base in *OsSPL17* is shown in lowercase. The OsmiR156 binding site in *OsSPL17* is highlighted in red. **b,** Sequencing chromatogram of Line 3 at the potential off-target site 1 in *OsSPL17*. Arrow points to the position with an edited base.
Supplemental Table 1 The base editing windows of ABE-P1 and ABE-P1S at different target sites.

| sgRNA   | Target gene | Base editor | Genotype of mutations | Base editing positions |
|---------|-------------|-------------|-----------------------|------------------------|
| sgRNA1  | OsSPL14     | ABE-P1      | T_{r-C}(6), T_{r-C}C_{6}(4), T_{r-T-C}C_{6}(6), T_{r-T-T-C}C_{6}(2) | 5, 7, 10               |
|         |             | ABE-P1S     | T_{r-C}(1), T_{r-T-C}C_{5}(5), T_{r-T-T-C}C_{5}(1), T_{r-T-T-T-C}C_{5}(6) | 1, 3, 5, 7, 10, 12     |
|         |             |             | T_{r-T-T-T-T-C}C_{5}(1), T_{r-T-T-T-T-T-C}C_{5}(1) |                        |
| sgRNA2  | SLR1        | ABE-P1      | T_{r-C}(7)           | 6                      |
|         |             | ABE-P1S     | T_{r-C}(7)           | 6                      |
| sgRNA3  | OsSerk2     | ABE-P1      | T_{r-C}(21), T_{r-T-C}C_{15}(5) | 6, 8                   |
|         |             | ABE-P1S     | T_{r-C}(11), T_{r-T-C}C_{10}(10) | 6, 8                   |
| sgRNA4  | Tms9.1      | ABE-P1      | T_{r-C}(1)           | 5                      |
|         |             | ABE-P1S     | T_{r-C}(4)           | 5                      |
| sgRNA5  | OsNRT1.1B   | ABE-P1      | A_{r-G}(1), A_{r-G}(2), A_{r-G}(6), A_{A-A-G-G-G-G}(1), A_{A-A-G-G-G-G}(15), A_{A-A-A-A-G-G-G-G-G-G}(1) | 4, 6, 8, 12            |
|         |             | ABE-P1S     | A_{r-G}(4), A_{r-G}(2), A_{A-A-G-G-G-G}(1), A_{A-A-G-G-G-G}(3), A_{A-A-G-G-G-G}(9), A_{A-A-A-G-G-G-G-G-G-G-G}(1) | 4, 6, 8, 12            |
| sgRNA6  | OsACC1      | ABE-P1      | T_{r-C}(6), T_{r-C}(5), T_{r-T-C}C_{14}(14) | 4, 7                   |
|         |             | ABE-P1S     | T_{r-C}(1), T_{r-C}(20), T_{r-T-C}C_{1}(1), T_{r-T-T-C}C_{9}(1), T_{r-T-T-T-C}C_{9}(1) | 2, 4, 7               |
| sgRNA7  | OsDEP1      | ABE-P1      | A_{r-G}(1), A_{r-G}(1), A_{A-A-G-G-G-G-G-G}(30), A_{A-A-A-G-G-G-G-G-G}(8) | 3, 5, 6               |
|         |             | ABE-P1S     | A_{A-A-G-G-G-G-G-G}(21), A_{A-A-A-G-G-G-G-G-G-G-G-G}(4) | 3, 5, 6               |

Note: For the base editors ABE-P1 and ABE-P1S, the base editing position was counted from the PAM-distal end, scoring the PAM sequence as positions 21-23.
## Supplemental Table 2

The base editing windows of ABE-P2, ABE-P2S, ABE-P5 and ABE-P5S at different target sites.

| sgRNA   | Target gene | Base editor | Genotype of mutations                                                                 | Base editing positions |
|---------|-------------|-------------|---------------------------------------------------------------------------------------|------------------------|
| sgRNA8  | SPR-MSF2    | ABE-P2      | T_{C}C_{1}, T_{C}C_{2}, T_{C}C_{3}, T_{C}C_{4}                                        | 1, 9, 15               |
|         |             | ABE-P2S     | T_{C}C_{1}, T_{C}C_{2}, T_{C}C_{3}, T_{C}C_{4}, T_{C}C_{5}, T_{C}C_{6}C_{7}C_{8}      | 3, 6, 9, 12, 15        |
| sgRNA9  | OsSPL14     | ABE-P2      | T_{C}C_{1}, T_{C}C_{2}, T_{C}C_{3}, T_{C}C_{4}, T_{C}C_{5}, T_{C}C_{6}C_{7}C_{8}      | 6, 8, 10, 14           |
|         |             |             | T_{C}C_{9}C_{10}C_{11}                                                               |                        |
|         | OsSPL17     | ABE-P2      | T_{C}C_{1}, T_{C}C_{2}, T_{C}C_{3}, T_{C}C_{4}, T_{C}C_{5}, T_{C}C_{6}C_{7}C_{8}      | 6, 8, 10, 12, 14       |
|         |             |             | T_{C}C_{9}C_{10}C_{11}                                                               |                        |
|         | OsSPL14     | ABE-P2S     | T_{C}C_{1}, T_{C}C_{2}, T_{C}C_{3}, T_{C}C_{4}, T_{C}C_{5}, T_{C}C_{6}C_{7}C_{8}      | 6, 8, 10, 12, 14, 17  |
|         |             |             | T_{C}C_{9}C_{10}C_{11}                                                               |                        |
|         | OsSPL17     | ABE-P2      | T_{C}C_{1}, T_{C}C_{2}, T_{C}C_{3}, T_{C}C_{4}, T_{C}C_{5}, T_{C}C_{6}C_{7}C_{8}      | 8, 10, 12, 14          |
|         |             |             | T_{C}C_{9}C_{10}C_{11}                                                               |                        |
|         | OsSPL16     | ABE-P2S     | T_{C}C_{1}, T_{C}C_{2}, T_{C}C_{3}, T_{C}C_{4}, T_{C}C_{5}, T_{C}C_{6}C_{7}C_{8}      | 6, 8, 10, 12, 14       |
|         |             |             | T_{C}C_{9}C_{10}C_{11}                                                               |                        |
|         | OsSPL18     | ABE-P2      | T_{C}C_{1}, T_{C}C_{2}, T_{C}C_{3}, T_{C}C_{4}, T_{C}C_{5}, T_{C}C_{6}C_{7}C_{8}      | 8, 10, 12, 14, 17      |
|         |             |             | T_{C}C_{9}C_{10}C_{11}                                                               |                        |
|         | OsSPL13     | ABE-P5      | T_{C}C_{1}                                                               | 11                     |
|         |             | ABE-P5S     | T_{C}C_{1}, T_{C}C_{1}                                                               | 7, 9                   |
| sgRNA12 | SNB         | ABE-P5      | A_{G}G_{1}                                                               | 4                      |
|         |             | ABE-P5S     | A_{G}G_{1}, A_{G}G_{2}, A_{G}G_{3}                                                              | 4, 8, 9                |

Note: For the base editors ABE-P2, ABE-P2S, ABE-P5 and ABE-P5S, the base editing position was counted from the PAM-distal end, scoring the PAM sequence as positions 22-27.
Supplemental Table 3  The base editing frequencies of ABE-P1 and ABE-P1S at potential off-target sites of sgRNA1.

| Site         | Chr | Position  | Guide-PAM sequence       | Base editor | Mismatch numbers | Number of genotyped lines | Number of edited lines | Editing efficiency |
|--------------|-----|-----------|--------------------------|-------------|------------------|---------------------------|-----------------------|-------------------|
| On-target    | 8   | 25275163  | AGAGAGAGCACAGCTCAGTGG    | ABE-P1      | 0                | 48                        | 18                    | 37.5%             |
|              |     |           |                          | ABE-P1S     | 0                | 17                        | 12                    | 70.6%             |
| Off-target 1 | 9   | 19910318  | AGAGAGAGCACAGCTCAGTGG    | ABE-P1      | 1                | 8                         | 0                     | 0                 |
|              |     |           |                          | ABE-P1S     | 1                | 8                         | 1                     | 12.5%             |
| Off-target 2 | 1   | 24179644  | sGAsGpGCACAGCTCAGTGG     | ABE-P1      | 5                | 8                         | 0                     | 0                 |
|              |     |           |                          | ABE-P1S     | 5                | 8                         | 0                     | 0                 |
| Off-target 3 | 3   | 36157080  | AGAsAGAGCAGpGTCAGTGG     | ABE-P1      | 4                | 8                         | 0                     | 0                 |
|              |     |           |                          | ABE-P1S     | 4                | 8                         | 0                     | 0                 |
| Off-target 4 | 3   | 8596704   | AGAGAGAGCACAGCGpGAGpGCG  | ABE-P1      | 4                | 8                         | 0                     | 0                 |
|              |     |           |                          | ABE-P1S     | 4                | 8                         | 0                     | 0                 |
| Off-target 5 | 4   | 23025825  | sGAsGpGCpGpGTCCTAGpGCG  | ABE-P1      | 5                | 8                         | 0                     | 0                 |
|              |     |           |                          | ABE-P1S     | 5                | 8                         | 0                     | 0                 |
| Off-target 6 | 5   | 10284901  | AGAGGCAGpGTCAGTGG        | ABE-P1      | 4                | 8                         | 0                     | 0                 |
|              |     |           |                          | ABE-P1S     | 4                | 8                         | 0                     | 0                 |
| Off-target 7 | 7   | 19030423  | AGAGAGAGCAGpGTCAGTGG     | ABE-P1      | 4                | 8                         | 0                     | 0                 |
|              |     |           |                          | ABE-P1S     | 4                | 8                         | 0                     | 0                 |
| Off-target 8 | 10  | 1012640   | AGAGAGAGCAGpGTCAGpGCG    | ABE-P1      | 4                | 8                         | 0                     | 0                 |
|              |     |           |                          | ABE-P1S     | 4                | 8                         | 0                     | 0                 |
| Off-target 9 | 7   | 20160420  | AGAsAGAGCAGCAGpGAGpGCG   | ABE-P1      | 4                | 8                         | 0                     | 0                 |
|              |     |           |                          | ABE-P1S     | 4                | 8                         | 0                     | 0                 |

Note: Nucleotides of PAM sequence are written in bold, and the mismatch bases in potential off-targets are shown in lowercase.
**Supplemental Table 4** The base editing frequencies of ABE-P1 and ABE-P1S at potential off-target sites of sgRNA6.

| Site     | Chr | Position   | Guide-PAM sequence                  | Base editor | Mismatch numbers | Number of genotyped lines | Number of edited lines | Editing efficiency |
|----------|-----|------------|-------------------------------------|-------------|------------------|----------------------------|-----------------------|-------------------|
| On target | 5   | 13065463   | CCCAGAACCATTAGTTGCTATG               | ABE-P1      | 0                | 36                         | 25                    | 69.4%             |
| On target | 5   | 13065463   | CCCAGAACCATTAGTTGCTATG               | ABE-P1S     | 0                | 42                         | 31                    | 73.8%             |
| Off-target 1 | 8   | 22061977   | CATAGCACTCAAtCAGTTGGG               | ABE-P1      | 4                | 8                          | 0                     | 0                 |
| Off-target 1 | 8   | 22061977   | CATAGCACTCAAtCAGTTGGG               | ABE-P1S     | 4                | 8                          | 0                     | 0                 |
| Off-target 2 | 5   | 17365477   | aTAGCACTCAaTGGTGCTG                 | ABE-P1      | 4                | 8                          | 0                     | 0                 |
| Off-target 2 | 5   | 17365477   | aTAGCACTCAaTGGTGCTG                 | ABE-P1S     | 4                | 8                          | 0                     | 0                 |
| Off-target 3 | 3   | 26476439   | CATAGCACTAAGGGGCTGAG               | ABE-P1      | 4                | 8                          | 0                     | 0                 |
| Off-target 3 | 3   | 26476439   | CATAGCACTAAGGGGCTGAG               | ABE-P1S     | 4                | 8                          | 0                     | 0                 |

Note: Nucleotides of PAM sequence are written in bold, and the mismatch bases in potential off-targets are shown in lowercase.

**Supplemental Table 5** The base editing frequencies of ABE-P2S at potential off-target sites of sgRNA9.

| Site     | Chr | Position   | Guide-PAM sequence                  | Mismatch numbers | Number of genotyped lines | Number of edited lines | Editing efficiency |
|----------|-----|------------|-------------------------------------|------------------|----------------------------|-----------------------|-------------------|
| On target 1 | 8   | 25275156   | ACAGAAGAGAGAGACACGCTCGAGT           | 0                | 36                         | 20                    | 55.6%             |
| On target 2 | 9   | 18918911   | ACAGAAGAGAGAGACACGCTCGAGT           | 0                | 36                         | 22                    | 61.1%             |
| Off-target 1 | 9   | 19647839   | ACAGAAGAGAGAGACACGCTCGAGT           | 2                | 8                          | 0                     | 0                 |
| Off-target 2 | 11  | 17631827   | ACAGAAGAGAGAGACACGCTCGAGT           | 2                | 8                          | 0                     | 0                 |
| Off-target 3 | 8   | 26505555   | ACAGAAGAGAGAGACACGCTCGAGT           | 2                | 8                          | 0                     | 0                 |
| Off-target 4 | 3   | 36137083   | ACgGAAGAGAaAGAGCAagTGGAGT           | 5                | 8                          | 0                     | 0                 |

Note: Nucleotides of PAM sequence are written in bold, and the mismatch bases in potential off-targets are shown in lowercase.
### Supplemental Table 6
The target sites and primers for all sgRNAs used in this study.

| sgRNA     | Target site                      | Forward primer 5'-3'            | Reverse Primer 5'-3'         |
|-----------|----------------------------------|----------------------------------|------------------------------|
| sgRNA1    | AGAGAGAGACACAGCTCAGT            | TGTGAGAGAGACACAGCTCAGT          | AACAACCTGAGCTGTCCTCCTCTCTCT |
| sgRNA2    | AGTGCACGGTGTCCGTGGCC            | TGTGAGTGCACGGTGTCCGTGGCC        | AAACGCGACAGACCCGAGCGACTGACTG |
| sgRNA3    | GGGGCAGCAGCTCATTGCC             | TGTGGGGCAGCAGCTCATTGCC         | AAACCGACAGAGACCCGAGCGACTGAG |
| sgRNA4    | AGGAGAGGCTGACAGCTTG             | TGTGAGAGGCTGACAGCTTG           | AACAACAGGACTGCTCCTCTGAGGCG  |
| sgRNA5    | ACTAGATATCTAACAACCTTA           | TGTGACTAGATATCTAACAACCTTA      | AACAATGTTAGAATCTACAGGACTGAG |
| sgRNA6    | CATAGCACATCAATGGCAGTCT          | TGTGACTAGACATCAATTGGGTCTCTTCT  | AAACAGAGAAGCTGATAGCTACTGACT |
| sgRNA7    | AGACAGACGCTGGCCCTTTT            | TGTGAGACAGACGCTGGCCCTTTT       | AAACAAAGAGGGCCAAGCTTGTCTCA  |
| sgRNA8    | AGATGACCATCAACAAATCCA           | TGTGAGATGACCATCAACAAATCCA      | AAACATGGATTGTAGTGTGTCACTTCA |
| sgRNA9    | ACAGAAGAGAGAGACACAGG            | TGTGAGACAGACGAGAGACACAGG       | AAACACCGTGGTTCCTCTCTGAGGAC  |
| sgRNA10   | AGTGGCCCTCTTTTGTGACTGAGGGCAGT   | TGTGAGTGGCCCTCTTTTGTGACTGAGGGCAGT | AAACAAATGGTTAGAATCTACAGGACTG |
| sgRNA11   | AGGAGCTGAGAGACAGATGCC           | TGTGAGAGCTGAGAGACAGATGCC       | AAACAGAGAAGCTGATAGCTACTGACT |
| sgRNA12   | CGTAGAGAATCTCAGATAGT            | TGTGAGCTAGAGAATCTCAGATAGT      | AAACAGACAGACGAGAGACAGGACTGAG |

### Supplemental Table 7
Primers for on-target site amplification and sequencing.

| Primer name          | Primer sequence 5'-3' | Purpose                          |
|----------------------|-----------------------|----------------------------------|
| OsSPL14-F            | AGGGTTCCAACAGCAGCGTAAGGA | Amplify all the OsSPL14 target sites |
| OsSPL14-R            | TGGTGCTGGGCCAGCGTGGCTCTCT  |                                    |
| OsSPL14seq-F         | TCTCCGCTGGTATCCAGTGGCAG  | Sanger sequencing primer          |
| SLR1-F               | GCGCAATTTATACGTGTACAGG  |                                    |
| SLR1-R               | AGCGGAGCGCTCAGCAGGTCAGG  | Amplify the SLR1 target site      |
| SLR1seq-F            | CGTCGAGACCTGCTGTGAGGCC  | Sanger sequencing primer          |
| OsSERK2-F            | CCATCTGCAATAGCTAGTCTTTT  | Amplify the OsSERK2 target site   |
| OsSERK2-R            | AGAGTGTACGTACATACAGCAG  |                                    |
| OsSERK2seq-F         | GCTTCGGTGAGCAGTAAGG     | Sanger sequencing primer          |
| Tms9-1-F             | TGCCGGTCTGGACCGTGGCGC   | Amplify the Tms9-1 target site    |
| Tms9-1-R             | CACGAGGCTCAGCAGAATGTGC  |                                    |
| Tms9-1seq-F          | CTCCATGGCCGTCTGGTCTGGT  | Sanger sequencing primer          |
| OsNRT1.1B-F          | GGGAGTTCATAGTGTGGAACGAGC | Amplify the OsNRT1.1B target site |
| OsNRT1.1B-R          | GTGCTATGCGCCAGCGATGATCC |                                    |
| OsNRT1.1Bseq-F       | CTGTGACACTTGAGCAGCATGAC  | Sanger sequencing primer          |
| OsACC1-F             | GCAATCTGTTCCTGTGCTGGAGC  | Amplify the OsACC1 target site    |
| OsACC1-R             | CTTGAAAGCGATTTTGTGTCAGAC |                                    |
| OsACC1seq-F          | GGCAATCTGTTCCTGTGCTGGAGC | Sanger sequencing primer          |
| OsDEP1-F             | CTCTTTCACATTGCTGCTGCTGCT | Amplify the OsDEP1 target site    |
| OsDEP1-R             | AGCAGACAGACAGATGTGCAAGAC  |                                    |
| OsDEP1seq-F          | AGGAGATGCCCATAGCTGGGCGGC  | Sanger sequencing primer          |
| SPX-MSF2-F           | AGCACACACACAGACAGTGTGTC  | Amplify the SPX-MSF2 target site  |
| SPX-MSF2-R           | GCGCAACTTAGGTTGGCATTGCC  |                                    |
| SPX-MSF2seq-F        | CGTCTGACATGCGAGCCGCGT    | Sanger sequencing primer          |
| OsSPL17-F            | GGTTTCACAGCGATGTGAGAGGA  | Amplify the OsSPL17 target site   |
| OsSPL17-R            | GGACCTGCAGACGACAACC      |                                    |
### Table 8 Primers for off-target site amplification and sequencing.

| Primer name       | Primer sequence 5'-3' | Purpose                                      |
|-------------------|-----------------------|----------------------------------------------|
| sgRNA1M1-F        | GGGACCTCGTTGTCAGCAACAACCC | Amplify the off target site 1 for sgRNA1    |
| sgRNA1M1-R        | GCAGGTCCAGAAGCTTTGTGGA |                                            |
| sgRNA1M1seq-F     | CTCGCCGCAATGTTATTGCT  | Sanger sequencing primer                     |
| sgRNA1M2-F        | TTGTCTCCTGAAACAATCCCGAG | Amplify the off target site 2 for sgRNA1    |
| sgRNA1M2-R        | CCTGTTGTCAGAGTACAAATTG |                                            |
| sgRNA1M2seq-F     | GTCAACCCTGACAGGGACAG  | Sanger sequencing primer                     |
| sgRNA1M3-F        | TGCAATGCTTACCTCTGCTAG | Amplify the off target site 3 for sgRNA1    |
| sgRNA1M3-R        | GATCACACTAGCGACAGCGAGC |                                            |
| sgRNA1M3seq-F     | TTGGACGGAGCAAAATATAAG | Sanger sequencing primer                     |
| sgRNA1M4-F        | ACGAGAGCTTCAGCTGACAGCA | Amplify the off target site 4 for sgRNA1    |
| sgRNA1M4-R        | AATCTGCCGCGTGCTGACAGAG |                                            |
| sgRNA1M4seq-F     | CGCCTCGGGATACAGCAGAT  | Sanger sequencing primer                     |
| sgRNA1M5-F        | TGCAGGTGGTCGGCGATCGCG | Amplify the off target site 5 for sgRNA1    |
| sgRNA1M5-R        | ACGCGGCACGGTCTGAGCTTCTGCTTCT | Sanger sequencing primer                  |
| sgRNA1M5seq-F     | ACTACTGGAAACAGCACCTA |                                            |
| sgRNA1M6-F        | GATCACAACTGCTCGTGAAGCTA | Amplify the off target site 6 for sgRNA1    |
| sgRNA1M6-R        | ATATGTCTTATCCGAGACGAC |                                            |
| sgRNA1M6seq-F     | AGACGCAAGAGACAGACAAGC | Sanger sequencing primer                     |
| sgRNA1M7-F        | CCAGACCTACATTCTAGGCTTCTC | Amplify the off target site 7 for sgRNA1    |
| sgRNA1M7-R        | GAGTTGACAGAGGGAGAGAGA |                                            |
| sgRNA1M7seq-F     | CCAAAACCCCTAAATCGGGAATCT | Sanger sequencing primer                  |
| sgRNA1M8-F        | ATATGGTCAATCCCTGGTGAAGAGAGA | Amplify the off target site 8 for sgRNA1    |
| sgRNA1M8-R        | CCCCAACGCTCGGAACTCGGCA |                                            |
| sgRNA1M8seq-F     | GAGGGGAATACACAGGCTTCTC | Sanger sequencing primer                     |
| sgRNA1M9-F        | CAGAGCCTGGAGCGGTCTTCTAC | Amplify the off target site 9 for sgRNA1    |
| sgRNA1M9-R        | ACAGTGACAAAATCGACAGAGAGA |                                            |
| sgRNA1M9seq-F     | GAGTGGCAACCGCAGTGCTTCTC | Sanger sequencing primer                     |
| sgRNA   | Primer | Function                  |
|---------|--------|---------------------------|
| 6M1-F   | CTCAACTCTTGGGATTGCAGTTGC | Amplify the off target site 1 for sgRNA6 |
| 6M1-R   | CCGATAAGGTTTGCTATTCACAGG | Sanger sequencing primer |
| 6M1seq-F| GTAGCTAATGGTATTGTATAC    |                           |
| 6M2-F   | GGCACATGTTTTGTTCTCTCTGC | Amplify the off target site 2 for sgRNA6 |
| 6M2-R   | TTCCACTGAGATGGCTTGTGAC  | Sanger sequencing primer |
| 6M2seq-F| ATGCAATGAGAGGCATCAAGAG  |                           |
| 6M3-F   | CGAGAGAGTACAGCAGCAGCTG  | Amplify the off target site 3 for sgRNA6 |
| 6M3-R   | CTCCGCACGCCCTCGAGTTCTGCC | Sanger sequencing primer |
| 6M3seq-F| CCGGCACCATCTCAACCG      |                           |

| sgRNA   | Primer | Function                  |
|---------|--------|---------------------------|
| 9M1-F   | GCAACAAGATGTCTTCTCCCG  | Amplify the off target site 1 for sgRNA9 |
| 9M1-R   | CTGCCGCCGAACTGCTGCTGC | Sanger sequencing primer |
| 9M1seq-F| TGAACGAGCGAGCGACGAC  |                           |
| 9M2-F   | ACACCTGCCAAGAGAATGGCA | Amplify the off target site 2 for sgRNA9 |
| 9M2-R   | CCGATAAGCTGATCAGAACGC | Sanger sequencing primer |
| 9M2seq-F| GTGACAGCGGAAACCAAGAC |                           |
| 9M3-F   | AGACCAGCTGAGCTGCGGCG  | Amplify the off target site 3 for sgRNA9 |
| 9M3-R   | GAGACGGTACGTCCTCTCTC | Sanger sequencing primer |
| 9M3seq-F| GAAGACGGTAGCTCCTCC    |                           |
| 9M4-F   | TATATTCGTCGAAATGCATCTA| Amplify the off target site 4 for sgRNA9 |
| 9M4-R   | ATGCCAGCGGTTCTAACCAGCA |                           |
| 9M4seq-F| CATTCTCACTCTCCACTATTTCC| Sanger sequencing primer |