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CRISPR–Cas12a base editors confer efficient multiplexed genome editing in rice

Dear Editor,

Many Cas9-derived base editors have been developed for precise C–to–T and A–to–G base editing in plants (Molla et al., 2021). They are typically based on a SpCas9 nickase or its engineered variants with altered protospacer adjacent motif (PAM) requirements (Molla et al., 2021). CRISPR–Cas12a enables highly efficient multiplexed genome editing in plants, and its T-rich PAM preference complements the G–rich PAM requirement of SpCas9 in genome targeting (Zhang et al., 2019, 2021). Because of the lack of an efficient Cas12a nickase, it has been challenging to develop efficient Cas12a base editors. Nevertheless, Cas12a cytosine base editors (CBEs) and adenine base editors (ABEs) have been developed in mammalian cells (Li et al., 2018; Kleinstiver et al., 2019) with low DNA damage (Wang et al., 2020) because deactivated Cas12a (dCas12a) was used. However, efficient dCas12a base editors are yet to be developed in plants.

We reasoned that an efficient Cas12a base editor could be developed in plants if a high–processing deaminase was linked to an efficient dCas12a via an optimal linker. It was previously shown that cytidine deaminase hA3A-Y130F is highly efficient for Cas9D10A-mediated C–to–T base editing in plants (Li et al., 2021; Ren et al., 2021). We replaced the Cas9D10A part of this CBE system with deactivated versions of four efficient Cas12a proteins: FnCas12a (Endo et al., 2016), Mb2Cas12a (Zhang et al., 2021), LbCas12a (Tang et al., 2017), and LbCas12a–D156R (Kleinstiver et al., 2019), also known as ttLbCas12a (Schindele and Puchta, 2020). Using the dual RNA polymerase II (Pol II) expression system and a default linker 1 (Figure 1A), these four Cas12a CBEs were assessed at three target sites in stable transgenic T0 rice lines using next–generation sequencing (NGS). The CBE–based dLbCas12a–D156R outperformed other Cas12a CBEs at all three sites (Figure 1B), with a preferred substrate for cytosine base conversion at position 10 (C10) in the protospacer sequences. Sanger sequencing further confirmed the heterozygous or homozygous edits in some T0 lines (Supplemental Figure 1). Thus, this dLbCas12a–D156R CBE enables high-efficiency cytosine base editing in rice, consistent with the high nuclease activity of LbCas12a–D156R in *Arabidopsis* (Schindele and Puchta, 2020).

Encouraged by the promising data, we decided to further improve the system by testing different flexible linkers between hA3A–Y130F and dLbCas12a–D156R. We performed multiplexed editing with four crispr RNAs (crRNAs) targeting five sites using the dual Pol II promoter and the ribozyme processing system (Figure 1D) (Tang et al., 2017). Seven different flexible linkers, including the default linker 1, were compared (Figure 1E). The multiplexed CBE T-DNA expression vectors with different linkers were assessed in transgenic rice calli by NGS. At the TTTTG08 site, highly efficient base editing was observed for all Cas12a CBEs, especially with linkers 2 and 4 (Figure 1F). Lower C–to–T base editing efficiencies were seen at the other three target sites with all linkers (Supplemental Figure 2A–2C). The C–to–T editing frequency of site ATTC02 with a VTTV PAM was lower than 10% (data not shown). At these low-activity sites, only C10 was preferentially edited (Supplemental Figure 2D–2F). By contrast, the editing window was expanded to the C8 and C11 positions at the high-activity TTTTG08 site (Figure 1G). Further genotype analysis of TTTTG08 and ATTTC02 sites showed that the dCas12a-D156R CBE with linker 4 conferred more robust editing (Supplemental Figure 3).

Together, our data suggest that hA3A-Y130F-linker 4-dLbCas121-D156R is the most robust Cas12a CBE among all those tested. Considering that all four target sites with a TTTV (V = A, C, G) PAM could be efficiently edited by Cas12a nuclease (Zhang et al., 2021), it is striking to see drastic differences in C-to-T base editing efficiencies among these sites. This suggests that Cas12a nuclease activity cannot be used to predict Cas12a base-editing activity at the same target site. The editable cytosine depends upon the sequence context.

Next, we selected three top-performing Cas12a CBE systems (with linkers 2, 4, and 8) for analysis of byproducts and off-target effects. Insertion or deletion byproducts could be detected at the high–activity TTTTG08 site, albeit at low efficiency (Supplemental Figure 4A). At the three low–activity sites, no editing byproducts were detected (Supplemental Figure 4B–4D). Potential off-target sites were predicted using Cas–OFFinder, and crRNA–dependent off–target effects were analyzed in eight base-edited calli for the top three CBEs using NGS. No off–target editing was observed for any of the CBE systems (Supplemental Figure 5).

We next sought to develop efficient Cas12a ABEs for A–to–G base editing in plants. We fused the high–processing ecTadA8e (Richter et al., 2020) to dCas12a proteins and expressed the components with the dual Pol II promoter system (Figure 1H). With a default flexible linker 3 that connects ecTadA8e and dCas12a (Figure 1E), we compared four dCas12a proteins at three target sites in transgenic T0 rice lines. Consistent with the CBE data, dLbCas12a–D156R–based ABE showed the highest A–to–G base editing efficiencies at all three sites (Figure 1I). An editing window from A8 to A11 was identified (Figure 1J). We further investigated the editing events by Sanger sequencing (Supplemental Figure 6). Notably, dLbCas12a–D156R ABEs

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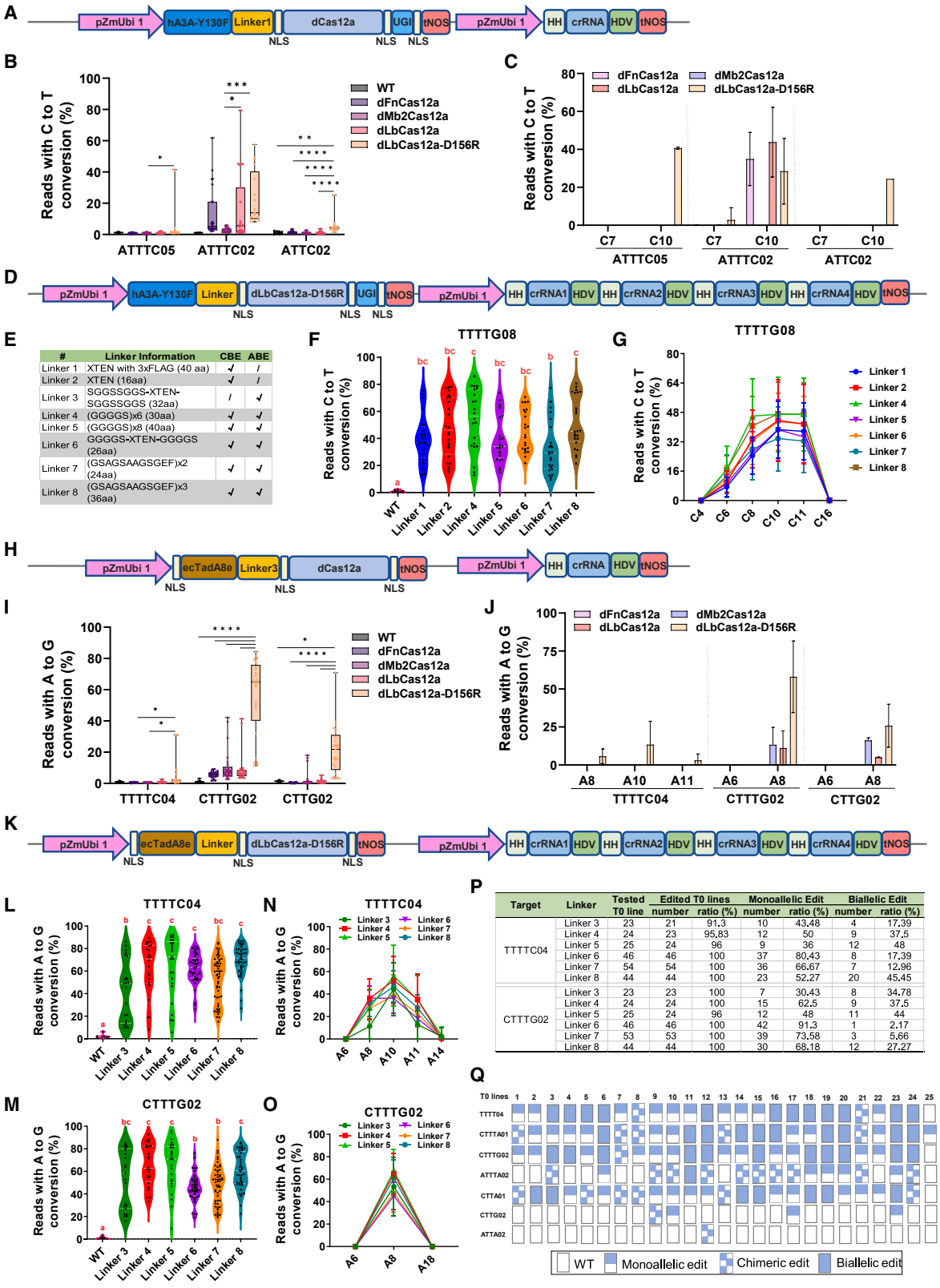

Figure 1. Development of CRISPR–Cas12a–based cytosine base editors (CBEs) and adenine base editors (ABEs) in rice.

(A) Schematic of the dual RNA polymerase II (Pol II) promoter system for dCas12a-based CBE and crRNA expression. pZmUbi1, maize ubiquitin promoter; NLS, nuclear localization signal; UGI, uracil DNA glycosylase; tNOS, nopaline synthase terminator; HH, hammerhead ribozyme; HDV, hepatitis delta virus ribozyme.

(legend continued on next page)

generated many monoallelic and biallelic edited lines at the CTTTG02 site. Moreover, 10 T0 plants were homozygous edited lines based on Sanger sequencing (Figures 1I and Supplemental Figure 6C). These data suggest that the dLbCas12a–D156R ABE is an efficient A–to–G base editing system in rice.

To further improve the dLbCas12a–D156R A–to–G editor, we compared six different flexible linkers (Figure 1E) with multiplexed editing using four crRNAs. Accordingly, six multiplexed LbCas12a-D156R ABEs with different linkers were constructed (Figure 1K). Because three of the four crRNAs (CA01, AA02, and CG02) can each target two sites with identical protospacers but different PAMs (TTTV or VTTV), a total of seven different sites in the rice genome were simultaneously targeted: four TTTV PAM sites and three VTTV PAM sites. Remarkably, high A–to–G editing was found at all four target sites with TTTV PAMs. The dLbCas12a–D156R ABEs with linkers 4, 5, and 8 outperformed ABEs with the other three linkers, generating higher enrichment of high–efficiency edited lines (Figures 1L and 1M and Supplemental Figures 7A and 7B). Consistently, these three dLbCas12a–D156R ABEs also produced more robust A–to–G base editing at the three target sites with non–canonical VTTV PAMs, especially at the CTTA01 site (Supplemental Figure 7C–7E). The editing window spanned from A8 to A12 in the protospacers (Figures 1N and 1O and Supplemental Figure 7F–7J). The dCas12a-D156R–based ABEs with linkers 4, 5, and 8 led to more robust A–to–G editing at the canonical TTTV PAM sites (Supplemental Figure 8).

We analyzed the genotypes of the resulting A–to–G editing lines at TTTTC04 and CTTTG02 sites. The data showed that dLbCas12a–D156R–based ABEs generated nearly 100% editing efficiency regardless of linkers (Figure 1P). Again, dLbCas12a-D156R-based ABEs with linkers 4, 5, and 8 stood out, as they led to high-frequency biallelic A–to–G base editing: 37.5%, 48%, and 45.45% at the TTTTC04 site and 37.5%, 44%, and 27.27% at the CTTTG02 sites, respectively (Figure 1P). We then expanded the genotype analysis to all seven target sites in 25 T0 lines edited by the efficient ecTadA8e–linker 5–dCas12a–D156R base editor. Impressively, five lines (#9, #10, #12, #17, and #23) showed simultaneous A–to–G editing at six target sites. Also, 10 T0 lines carried biallelic edits for at least three target sites, and two lines (#19 and #23) carried biallelic edits at four target sites (Figure 1Q and Supplemental Figure 9). Hence, multiplexed biallelic editing can be achieved within one generation using this ABE. Finally, we investigated the byproducts and crRNA–dependent off–target effects of these three top ABE systems. No significant byproduct editing and off–target editing were observed in the edited T0 lines examined (Supplemental Figures 10 and 11).

In this study, we developed highly efficient Cas12a CBEs and ABEs for multiplexed genome editing in plants. With less potential to generate DNA breaks compared with Cas9 nickase–based base editors, these T–rich PAM–targeting dCas12a base editors are very suitable for multiplexed promoter editing to fine–tune gene expression without generating insertion or deletion mutations. Our success is based on the combination of an efficient CRISPR–Cas12a expression system, a high–efficiency LbCas12a–D156R variant, high–activity cytidine and adenine deaminases, and optimal linkers. The optimized Cas12a CBEs produced highly efficient monoallelic editing at high–activity target sites. Significantly, the dLbCas12a–D156R ABEs developed in this study appear to be as efficient as the Cas9 ABEs (Molla et al., 2021). These efficient DNA break–free Cas12a CBE and ABE constructs have been deposited at Addgene and are promising tools for singular and multiplexed base editing in plants.

SUPPLEMENTAL INFORMATION

Supplemental information is available at *Plant Communications Online*.

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(B) Assessment of the C–to–T editing efficiency of four variants of dCas12a-based CBEs at sites ATTTC05, ATTTC02, and ATTC02 in transgenic rice plants.
(C) Editing window of four dCas12a-CBEs in transgenic rice plants at sites ATTTC05, ATTTC02 and ATTC02.
(D) Schematic of the dual Pol II promoter–based and multiplexed dLbCas12a-D156R CBE editing system.
(E) The list of linkers used between deaminases and dLbCas12a–D156R.
(F) Assessment of seven dLbCas12a–D156R CBEs with different linkers in regenerated rice calli at the TTTTG08 site. Each dot represents an independent callus. The first quartile, median, and third quartile are shown as dotted lines.
(G) Editing windows of seven dLbCas12a–D156R CBEs with different linkers in regenerated rice calli at the TTTTG08 site.
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(J) Editing window of four dCas12a– ABEs in transgenic rice plants at sites TTTTC04, CTTTG02, and CTTG02.
(K) Schematic of the dual Pol II promoter–based multiplexed dLbCas12a-D156R ABE editing system.
(L and M) Assessment of six dLbCas12a–D156R ABE editors with different linkers in transgenic rice plants at TTTTC04 and CTTTG02 sites. The first quartile, median, and third quartile are shown as dotted lines. Each dot represents an independent line.
(N and O) Editing windows of six dLbCas12a-D156R ABE editors with different linkers in transgenic rice plants at TTTTC04 and CTTTG02 sites.
(P) A–to–G base editing frequency of dLbCas12a–D156R–based ABEs at TTTTC04 and CTTTG02 sites.
(Q) Genotypes of 25 T0 lines edited by the dLbCas12a–D156R–linker 5–ABE at seven target sites. Wild type, chimeric edits, monoallelic edits, and biallelic edits are denoted as empty rectangles, dotted rectangles, half-filled rectangles, and fully filled rectangles, respectively.
To define genotypes in (**P**) and (**Q**): T0 lines with an A–to–G mutation frequency lower than 10% were regarded as wild type; T0 lines with an A–to–G mutation frequency from 10% to 30% were regarded as having chimeric edits; T0 lines with an A–to–G mutation frequency from 30% to 75% were regarded as having monoallelic edits; and T0 lines with an A–to–G mutation frequency higher than 75% were regarded as having biallelic edits. NGS of PCR amplicons was used to detect mutations in regenerated rice calli or T0 lines. All NGS data were analyzed with CRISPR RGEN tools. Data in (**C**), (**G**), (**J**), (**N**), and (**O**) are presented as mean values ± standard deviation. p values in (**B**) and (**I**) were obtained using a two-tailed Student's t-test: $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$, $^{****}p < 0.0001$. Different letters indicate significant differences ($p < 0.05$; one-way ANOVA, Tukey's test) in (**F**), (**L**), and (**M**).

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AUTHOR CONTRIBUTIONS

Y.C. and Y.Q. designed the experiments. Y.C., Y.Z., G.L., H.F., and S.S. generated the vectors. Y.C. performed rice transformation and NGS analysis for genome editing in transgenic rice calli and plants. Y.C. and A.F. performed Sanger sequencing of transgenic lines. J.X., J.L., and Q.Q. provided guidance on linkers for vector design. Y.C. and Y.Q. analyzed the data and wrote the manuscript. All authors participated in discussion and revision of the manuscript.

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Supplemental information

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CRISPR-Cas12a base editors confer efficient multiplexed genome editing in rice

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Supplemental Materials and Methods

Vector construction

The rice codon-optimized pYPQ230 (Addgene #86210), pYPQ239 (Addgene #108859), pYPQ284 (Addgene #138116), and pYPQ230-D156R (Addgene #195366) were used to generate attL1-attL5 Cas12a entry clones used in this study. The mutations were introduced to pYPQ230 harboring LbCas12a, pYPQ239 harboring FnCas12a, pYPQ284 harboring Mb2Cas12a, and pYPQ230-D156R harboring engineered LbCas12a-D156R (also known as ttLbCas12a) to generate dLbCas12a (D831A), dFnCas12a (D917A), dMb2Cas12a (D864A), and dLbCas12a-D156R (D831A) using the NEBuilder HiFi DNA Assembly Cloning Kit (New England Biolabs®) or Q5® Site-Directed Mutagenesis Kit (New England Biolabs®). To generate the Cas12a-based CBE vectors, PCR-amplified backbones from pYPQ265E2 (Addgene #164719) using corresponding dCas12a-BB primers, and insertion fragments from dCas12a with corresponding dCas12a-INS primers by Q5® High-Fidelity DNA Polymerase (New England Biolabs®) were used for assembling dLbCas12a-CBE, dFnCas12-CBE, dMb2Cas12a-CBE, and dLbCas12a-D156R-CBE through HiFi cloning. To generate the Cas12a-based ABE vectors, the dzSpCas9 in pYPQ262-ABE8e (Addgene #138116) was replaced by dCas12a at the BsrGI and NcoI sites to produce dLbCas12a-ABE, dFnCas12-ABE, dMb2Cas12a-ABE, and dLbCas12a-D156R-ABE by T4 ligation. To prepare dCas12a-D156R base editors with different linkers, the linkers of (GGGGS)x6, (GGGGS)x8, GGGGS-XTEN-GGGGS, (GSAGSAAGSGEF)x2, and (GSAGSAAGSGEF)x3 with overlapping sequences to the C-terminus of hA3A/Y130F and N-terminus of LbCas12a were synthesized as gBlocks® by IDT. The dLbCas12a-D156R, hA3A-Y130F, and UGI components were amplified from the dLbCas12a-D156R-CBE vector. Then, the purified PCR fragment was assembled with the linker using HiFi DNA Assembly mix to make CBE vectors with different linkers. The ABE vectors with different linkers were made in a similar way. Taking the dLbCas12a-D156R-Linker 5-ABE as an example, two PCR-amplified DNA fragments were prepared with primers ttLb-ABE-BB-L5_F and ttLb-ABE-BB-L5_R, and ttLb-ABE-L5_F and ttLb-ABE-L5_R, from dLbCas12a-D156R-ABE and (GGGGS)x8 gBlock, respectively, and then assembled using the HiFi cloning method. The other ABE editors were obtained using the corresponding primers and assembling by HiFi. All primers and gBlocks® used in this research are listed in Supplemental Table 1.

All target sites (Supplemental Table 3) were synthesized as single-strand oligonucleotides by GeneWiz. The forward and reverse primers were phosphorylated, annealed, and ligated at the BsmBI site with Instant Sticky-end Ligase Master Mix (New England Biolabs®) into pYPQ141-ZmUBI-RZ-Lb (Addgene #86197) for crRNA expression to be coupled with dLbCas12a and dLbCas12a-D156R base editors, or pYPQ141-ZmUBI-RZ-Fn (Addgene #108864) for crRNA expression to be coupled with dFnCas12a and dMb2Cas12a base editors. To express four crRNAs in the attL5-attL2 entry vectors, four tandem HH-crRNA-HDV were used based on the previously reported method (Zhang et al., 2021). Briefly, the four annealed crRNA protospacer sequences were ligated to pYPQ131-STU-Lb (Addgene #138096), pYPQ132-STU-Lb (Addgene #138099), pYPQ133-STU-Lb (Addgene #138102), and pYPQ134-STU-Lb (Addgene #138105) at the BsmBI site, respectively. The four crRNA cassettes were assembled into

the attL5-attL2 vector pYPQ144-ZmUBI-pT (Addgene #138108) using Golden Gate assembly.

The three-way Gateway LR reaction was used to assemble the T-DNA expression vector with an attR1-attR2 destination vector pYPQ203 (Addgene # 86207), an attL5-attL2 crRNA entry clone, and an attL1-attR5 base editor entry clone. All T-DNA vectors used in this study are listed in Supplemental Table 2.

Agrobacterium-mediated transformation of rice callus cells

The *Japonica* cultivar Kitaake rice was used in this study. *Agrobacterium*-mediated transformation was performed as published previously (Zhong et al., 2021). In brief, healthy and sterilized rice seeds were transferred to the plate with rice callus induction medium for 14 days under light. Calli in good condition were cut from the mature seeds and used for *Agrobacterium* infection. The T-DNA vectors were transformed into *Agrobacterium tumefaciens* strain EHA105 (OD_{600}=0.5) containing 100 uM acetosyringone. After three to five days of co-cultivation at 25 °C in the dark, the calli were washed completely to remove the residual *Agrobacterium*. Then, the calli were transferred to the selection medium containing 50 mg/L hygromycin for two weeks and then to fresh selection medium for another two weeks in the light at 32°C. White actively growing calli on the selection medium were transferred to the regeneration medium I and incubated at 29 °C (16h/8h light/dark). After two to three weeks, small seedlings were regenerated and transferred to regeneration medium II (in a single glass tube). The newly formed calli on regeneration medium I and leaf tissue from the regenerated T0 seedling were collected for DNA extraction and genotyping.

Off target analysis

The crRNA-dependent potential off-target sites were predicted using the online tool Cas-OFFinder (http://www.rgenome.net/cas-offinder/) (Bae et al., 2014). There were no potential off-target sites containing 1-3 nt mismatches found. Therefore, we examined 1 to 2 top off-target sites with 4-5 mismatches for each crRNA used in this study to check for off-target effects. The off-target sites were list in Supplemental Table 4. We picked the three top-performing systems of CBEs and ABEs, and selected 8 individual well-edited calli or T0 rice lines of each system for off-target analysis.

NGS sequencing and data analysis

The genomic DNA of regenerated rice calli or seedlings was extracted using the CTAB method (Stewart and Via, 1993) for PCR amplification and sequencing. For base editing with different Cas12a variants, there were 22 to 42 T0 rice lines genotyped for editing outcomes at three target sites. For dLbCas12a-D156R CBEs with seven different linkers, there were 22~24 transgenic calli genotyped for editing outcomes at five target sites. For dLbCas12a-D156R ABEs with six different linkers, there were 22~54 independent T0 rice lines genotyped for editing outcomes at five target sites.

For Sanger sequencing, the target regions were amplified with the Sanger sequencing primers. The PCR product was mixed with 5 uL of sequencing primer after the PCR products were treated with ExoI (New England Biolabs®) and Quick-CIP (New England Biolabs®), and then sent to GeneWiz for sequencing. The results were analyzed by Snapgene. For Next-generation sequencing (NGS), there were two rounds of PCR. Firstly, the target regions were amplified using target-specific primers with bridge sequences for barcoding primers to bind, and then 2 uL products from the 1st round of PCR were used as the template for the 2nd round PCR using the HiTom barcoding primers (Liu et al., 2019). About 20 to 30 of different PCR products were pooled together and purified by column. Purified PCR products (smaller than 500 bp) with partial Illumina® adapter sequences were normalized to a concentration of 20 ng/μl and in total at least 500 ng double-stranded DNA were prepared for NGS (GeneWiz, USA). The 2x250 bp sequencing without fragmenting the amplicons was performed. The NGS data was analyzed by CRISPR Rgen BE analyzer (http://www.rgenome.net/be-analyzer/#!).

All the statistical data were calculated using GraphPad Prism v.9.4.1. The multiple comparisons were performed according to one-way ANOVA and Tukey's multiple comparisons test.

Data availability

The seven Gateway® compatible Cas vectors used in this study have been deposited to Addgene: pYPQ230-D156R (ttLbCas12a) (#195366); dpYPQ230-D156R-CBE1 (hA3A/Y130F-linker 1-dLbCas12a-D156R-UGI, #195367); dpYPQ230-D156R-CBE2 (hA3A/Y130F-linker 2dLbCas12a-D156R-UGI, #195368); dpYPQ230-D156R-CBE4 (hA3A/Y130F-linker 4-dLbCas12a-D156R-UGI, #195369); dpYPQ230-D156R-ABE4 (ecTadA8e-linker 4-dLbCas12a-D156R, #195370); dpYPQ230-D156R-ABE5 (ecTadA8e-linker 5-dLbCas12a-D156R, #195371); dpYPQ230-D156R-ABE8 (ecTadA8e-linker 8-dLbCas12a-D156R, #195569).

The high-throughput sequencing data sets have been submitted to the National Center for Biotechnology information (NCBI) database under Sequence Read Archive (SRA) BioProject ID: PRJNA910189 for both on-target and off-target NGS sequencing data.

Reference:

Bae, S., Park, J. and Kim, J.S. (2014). Cas-OFFinder: a fast and versatile algorithm that searches for potential off-target sites of Cas9 RNA-guided endonucleases. *Bioinformatics* **30**: 1473-1475.

Liu Q, Wang C, Jiao X, Zhang H, Song L, Li Y, Gao C, Wang K. (2019). Hi-TOM: a platform for high-throughput tracking of mutations induced by CRISPR/Cas systems. *Sci China Life Sci.* **62**: 1-7.

Stewart, C.N., Jr., and Via, L.E. (1993). A rapid CTAB DNA isolation technique useful for RAPD fingerprinting and other PCR applications. *Biotechniques* **14**: 748-750.

Zhang Y, Ren Q, Tang X, Liu S, Malzahn AA, Zhou J, et al. (2021). Expanding the scope of plant genome engineering with Cas12a orthologs and highly multiplexable editing systems. *Nat Commun*. **12**:1944

SUPPLEMENTAL TABLES

Supplemental Table 1. Oligos and gBlocks used in this study

Name	Sequence 5'-3'	Description
Cpf1-OsROC5-gR1-F	TAGATTGCTTCCTGCAATGCCGGTAGAC	Forward primer for generating target ATTTC05
Cpf1-OsROC5-gR1-R	GGCCGTCTACCGGCATTGCAGGAAGCAA	Reverse primer for generating target ATTTC05
AC-crRNA2-F	TAGATGTCTCCCTGCAAACCACCGCGCA	Forward primer for generating target AC02
AC-crRNA2-R	GGCCTGCGCGGTGGTTTGCAGGGAGACa	Forward primer for generating target AC02
OsPDS-FokI-crRNA05-F	TAGATTTCAAAACCCTTAGAGATATCTA	Forward primer for generating target TTTTG05
OsPDS-FokI-crRNA05-R	GGCCTAGATATCTCTAAGGGTTTTGAAA	Forward primer for generating target TTTTG05
TTTN-OsmiR528-crRNA2-F	TAGATcctctctctCCTgtgcttgcctc	Forward primer for generating target TTTTG08
TTTN-OsmiR528-crRNA2-R	GGCCgaggcaagcacAGGagagagaggA	Forward primer for generating target TTTTG08
OsDEP1-cR2-23nt-F	tagatCAGAAAGAGAAGGAGGCACAGAT	Forward primer for generating target TTTTC04
OsDEP1-cR2-23nt-R	ggccATCTGTGCCTCCTTCTCTTTCTGa	Forward primer for generating target TTTTC04
CG-crRNA2-F	TAGATGCACCATATGCTTGCTGATCAAA	Forward primer for generating target CG02
CG-crRNA2-R	GGCCTTTGATCAGCAAGCATATGGTGCa	Forward primer for generating target CG02
AA-crRNA2-F	TAGATCCGGTGAAAAGGACCTTGTCCCA	Forward primer for generating target AA02
AA-crRNA2-R	GGCCTGGGACAAGGTCCTTTTCACCGGa	Forward primer for generating target AA02

CA-crRNA1-F	TAGATTACGTGGAAACAATGACAGTTCA	Forward primer for generating target CA01
CA-crRNA1-R	GGCCTGAACTGTCATTGTTTCCACGTAa	Forward primer for generating target CA01
dpYPQ230-HiFi-F1	cattgggattGCCCGGGGCGAGAGGAAC	Hifi primers for generating dLbCas12a
dpYPQ230-HiFi-R2	cgccccgggcAATCCCAATGACGTAAGG	Hifi primers for generating dLbCas12a
pUC origin-HiFi-R1	taggtatctcagttcggtgtaggtcgttcgctcc	Hifi primers for generating dLbCas12a
pUC origin-HiFi-F2	acaccgaactgagatacctacagcgtgag	Hifi primers for generating dLbCas12a
dpYPQ230-BB_F	cgtaaagcatAAGCGGCCAGCGGCGACG	Preparation of dLbCas12a C to T base editor
dpYPQ230-BB_R	cgagctttgaAGCAGCTGGCACCCCGTG	Preparation of dLbCas12a C to T base editor
dpYPQ230-ins_F	gccagctgctTCAAAGCTCGAGAAATTC	Preparation of dLbCas12a C to T base editor
dpYPQ230-ins_R	ctggccgcttATGCTTTACGGATGTCTG	Preparation of dLbCas12a C to T base editor
Nos-term-R2	aatcatcgcaagaccggcaacagg	Preparation of dLbCas12a C to T base editor
FnC12aD917A-F	GAGAGCGCCACTTGGCATAC	Preparation of dFnCas12a
FnC12aD917A-R	CCCTGgCGATCGACAGAATG	Preparation of dFnCas12a
Mb2C12aD864A-F	CATAGcTCGGGGAGAAAGG	Preparation of dMb2Cas12a
Mb2C12aD864A-R	CCTATGACATTGACTTCATC	Preparation of dMb2Cas12a
dFnC12a-ins_F	gtgccagctgctTCGATCTATCAAGAATTCGTTAATAAG	Preparation of dFnCas12a C to T base editor
dFnC12a-ins_R	cgctggccgcttGTTATTCCTGTTCTGAACAAATTC	Preparation of dFnCas12a C to T base editor

dFnC12a-BB_F	gaacaggaataacAAGCGGCCAGCGGCGACGAA	Preparation of dFnCas12a C to T base editor
dFnC12a-BB_R	cttgatagatcgaAGCAGCTGGCACCCCGTGGATG	Preparation of dFnCas12a C to T base editor
dMb2C12a-INS_F	gtgccagctgctCTGTTTCAAGATTTTACACATC	Preparation of dMb2Cas12a C to T base editor
dMb2C12a-INS_R	cgctggccgcttTCTATTTTGAGCGAAATTCAAC	Preparation of dMb2Cas12a C to T base editor
dMb2C12a-BB_F	cgctcaaaatagaAAGCGGCCAGCGGCGACGAA	Preparation of dMb2Cas12a C to T base editor
dMb2C12a-BB_R	aatcttgaaacagAGCAGCTGGCACCCCGTGGATG	Preparation of dMb2Cas12a C to T base editor
HiTom-A5-F2	gagttggatgctggatggTCTGACATGTTCTCGTGCAT	HiTom primer for target ATTTC05
Hi-TOM-A5-R2	ggagtgagtacggtgtgcCACTGTTGATCAGCACTCAC	HiTom primer for target ATTTTC05
HiTom-AC02-TTTV-F2	ggagtgagtacggtgtgcTGCTCCCTTGGGGTGTTCGT	HiTom primer for target AC02-TTTV
HiTom-AC02-TTTV-R2	gagttggatgctggatggCCTCTGCAGCCTGGTAAGCA	HiTom primer for target AC02-TTTV
HiTom-D5-F	gagttggatgctggatggGGAGATTGGTATGAAACTGGGC	Hi-TOM primer for target TTTTG05
HiTom-D5-R	ggagtgagtacggtgtgcCCATGTGTGTTAATCCTCAGAC	Hi-TOM primer for target TTTTG05
Hi-TOM-L8-F	ggagtgagtacggtgtgcTTTGGTTTGGGATAGGTAGGTG	HiTom primer for target TTTTG08
Hi-TOM-L8-R	gagttggatgctggatggTTTCTCCTACCGCTGCTGAT	HiTom primer for target TTTTG08
HiTom-A4-F	ggagtgagtacggtgtgcCATTTTAAGCATGTTCGCTG	HiTom primer for target TTTTC04
HiTom-A4-R	gagttggatgctggatggGATCAAAACATACCCGATCC	HiTom primer for target TTTTC04

Hi-TOM-AA2-F	ggagtgagtacggtgtgcCCCTGAAAAACAAAATGACCA	HiTom primer for target AA02-TTTV
Hi-TOM-AA2-R	gagttggatgctggatggCGAGTTTGCACCGTCAAC	HiTom primer for target AA02-TTTV
Hi-TOM-CA1-F	ggagtgagtacggtgtgcAGAGGAGAGCTGCATCCTGA	HiTom primer for target CA01-TTTV
Hi-TOM-CA1-R	gagttggatgctggatggCAGAATGGAATTTTGATTAAAAAGG	HiTom primer for target CA01-TTTV
Hi-TOM-CG2-TTTV-F	ggagtgagtacggtgtgcAAGACAGATTCCTCTGCATTGA	HiTom primer for target CG02-TTTV
Hi-TOM-CG2-TTTV-R	gagttggatgctggatggGCGTCAAACTTTCCTTAACAGG	HiTom primer for target CG02-TTTV
HiTom-CG02-TTN-F	ggagtgagtacggtgtgcTTACAGTGGTGCAGTCTGT	HiTom primer for target CG02-TTN
HiTom-CG02-TTN-R	gagttggatgctggatggGAATACAAAGAAATAACAAAATGCAAG	HiTom primer for target CG02-TTN
HiTom-AC02-TTN-F	ggagtgagtacggtgtgcTGCTTGGCATCGTTGCTCTAT	HiTom primer for target AC02-TTV
HiTom-AC02-TTN-R	gagttggatgctggatggcgcctcatgctccatcact	HiTom primer for target AC02-TTV
Hi-TOM-AA2-TTN-F	ggagtgagtacggtgtgcGCCACCGCTAAGTTCCTCG	HiTom primer for target AA02-TTV
Hi-TOM-AA2-TTN-R	gagttggatgctggatggGTCGAAGATGCAGGGGCAC	HiTom primer for target AA02-TTV
Hi-TOM-CA1-TTN-F	ggagtgagtacggtgtgcGAAAGCAGAGGAGAGCTGCG	HiTom primer for target CA01-TTV
Hi-TOM-CA1-TTN-R	gagttggatgctggatggGAAGGGACTGATTAGTGCCAAA	HiTom primer for target CA01-TTV
Hi-TOM-F-1-correct	ACACTCTTTCCCTACACGACGCTCTTCCGATCTgcttGCGTtggagtgagtacggtgtgc	Hi-TOM barcoding primer

Hi-TOM-F-2-correct	ACACTCTTTCCCTACACGACGCTCTTCCGATCTgcttGTAGtggagtgagtacggtgtgc	Hi-TOM barcoding primer
Hi-TOM-F-3-correct	ACACTCTTTCCCTACACGACGCTCTTCCGATCTgcttACGCtggagtgagtacggtgtgc	Hi-TOM barcoding primer
Hi-TOM-F-4-correct	ACACTCTTTCCCTACACGACGCTCTTCCGATCTgcttCTCGtggagtgagtacggtgtgc	Hi-TOM barcoding primer
Hi-TOM-F-5-correct	ACACTCTTTCCCTACACGACGCTCTTCCGATCTgcttGCTCtggagtgagtacggtgtgc	Hi-TOM barcoding primer
Hi-TOM-F-6-correct	ACACTCTTTCCCTACACGACGCTCTTCCGATCTgcttAGTCtggagtgagtacggtgtgc	Hi-TOM barcoding primer
Hi-TOM-F-7-correct	ACACTCTTTCCCTACACGACGCTCTTCCGATCTgcttCGACtggagtgagtacggtgtgc	Hi-TOM barcoding primer
Hi-TOM-F-8-correct	ACACTCTTTCCCTACACGACGCTCTTCCGATCTgcttGATGtggagtgagtacggtgtgc	Hi-TOM barcoding primer
Hi-TOM-F-9-correct	ACACTCTTTCCCTACACGACGCTCTTCCGATCTgcttATACtggagtgagtacggtgtgc	Hi-TOM barcoding primer
Hi-TOM-F-10-correct	ACACTCTTTCCCTACACGACGCTCTTCCGATCTgcttCACAtggagtgagtacggtgtgc	Hi-TOM barcoding primer
Hi-TOM-F-11-correct	ACACTCTTTCCCTACACGACGCTCTTCCGATCTgcttGTGCtggagtgagtacggtgtgc	Hi-TOM barcoding primer

Hi-TOM-F-12-correct	ACACTCTTTCCCTACACGACGCTCTTCCGATCTgcttACTAtggagtgagtacggtgtgc	Hi-TOM barcoding primer
Hi-TOM-R-A	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTctgtGCGTtgagttggatgctggatgg	Hi-TOM barcoding primer
Hi-TOM-R-B	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTctgtGTAGtgagttggatgctggatgg	Hi-TOM barcoding primer
Hi-TOM-R-C	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTctgtACGCtgagttggatgctggatgg	Hi-TOM barcoding primer
Hi-TOM-R-D	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTctgtCTCGtgagttggatgctggatgg	Hi-TOM barcoding primer
Hi-TOM-R-E	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTctgtGCTCtgagttggatgctggatgg	Hi-TOM barcoding primer
Hi-TOM-R-F	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTctgtAGTCtgagttggatgctggatgg	Hi-TOM barcoding primer
Hi-TOM-R-G	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTctgtCGACtgagttggatgctggatgg	Hi-TOM barcoding primer
Hi-TOM-R-H	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTctgtGATGtgagttggatgctggatgg	Hi-TOM barcoding primer
A5-NGS-F1	CCGTCCcatcatcgacaacagccttg	Sanger sequencing primer for target ATTTC05
A5-NGS-R1	TCATTCgcacctgtagctcagccttc	Sanger sequencing primer for target ATTTC05

AC02-TTTN-NGS-F1	ACAGTGgaaattcccgtgcgtttcagg	Sanger sequencing primer for target AC02-TTTV
AC02-TTTN-NGS-R	CAGACGGCTTGGATAAACTG	Sanger sequencing primer for target AC02-TTTV
AC02-TTN-Sanger-F	TCTCTCTCGCCGGATTGGAT	Sanger sequencing primer for target AC02-TTV
AC02-TTN-Sanger-R	TCGTTGAGCTCGTCGTCCTA	Sanger sequencing primer for target AC02-TTV
OsPDS-F3	aaatgccttgaacagATAGCTG	Sanger sequencing primer for target TTTTG05
OsPDS-R4	gctccatggcatgatctattg	Sanger sequencing primer for target TTTTG05
L8-sanger-F	GATCAGCAGCAGCCACAGCA	Sanger sequencing primer for target TTTTG08
L8-sanger-R	GTAGCGGCATTTCTCTGAAACA	Sanger sequencing primer for target TTTTG08
OsDep1-F5	GAACATGTGTGCTCAAATTG	Sanger sequencing primer for target TTTTC04
OsDep1-R4	CTACATGTAATTTCAGCCGCA	Sanger sequencing primer for target TTTTC04
CG02-TTTV-F	CGTGCTGAACGAGTAGAGCTT	Sanger sequencing primer for target CG02-TTTV
CG02-TTTV-R	CCTTCAGGCTGCAAATTGAT	Sanger sequencing primer for target CG02-TTTV
CG02-TTV-F	TCAAATTGCATTTGCGGTAA	Sanger sequencing primer for target CG02-TTV
CG02-TTV-R	CGCCAGCAAAAGCAATAACT	Sanger sequencing primer for target CG02-TTV
AA02-TTTN-sanger-F	AGATAAGCTCACTCTTGCCC	Sanger sequencing primer for target AA02-TTTV
AA02-TTTN-sanger-R	GACACTCCGAGTAGATGACA	Sanger sequencing primer for target AA02-TTTV

AA02-TTN-sanger-F	GGACAAGGGTAGTACACTGA	Sanger sequencing primer for target AA02-TTV
AA02-TTN-sanger-R	AATGCGACTCGGGTAGCAAA	Sanger sequencing primer for target AA02-TTV
CA01-TTTN-sanger-F	CTACTAGCACTGGTAGTGGA	Sanger sequencing primer for target CA01-TTTV
CA01-TTTN-sanger-R	GCACATATGAAAGGCCCATC	Sanger sequencing primer for target CA01-TTTV
CA01-TTN-sanger-F	GCATAGAGGTTGTACCACA	Sanger sequencing primer for target CA01-TTV
CA01-TTN-sanger-R	ATGATAGGCCCATCACATG	Sanger sequencing primer for target CA01-TTV
ttLb-ABE-BB-L4_F	CGGCGGGTCGACCATGGCTCCTAAGAAGAAG	Primers for dLbCas12a-D156R-ABE-L0
ttLb-ABE-BB-L4_R	CCCCGCCCCCGTTGATGGAGCTCTGGGC	Primers for dLbCas12a-D156R-ABE-L0
ttLb-ABE-L4_F	CTCCATCAACGGGGGCGGGGGCTCAGGC	Primers for dLbCas12a-D156R-ABE-L0
ttLb-ABE-L4_R	GAGCCATGGTCGACCCGCCGCCCCCGCT	Primers for dLbCas12a-D156R-ABE-L0
Lb-ABE-BB-L5_F	AGGAGGGAGCACCATGGCTCCTAAGAAGAAG	Primers for dLbCas12a-D156R-ABE-L1
Lb-ABE-BB-L5_R	CTCCCCCTCCGTTGATGGAGCTCTGGGC	Primers for dLbCas12a-D156R-ABE-L1
ttLb-ABE-L5_F	CTCCATCAACGGAGGGGGAGGGTCGGGA	Primers for dLbCas12a-D156R-ABE-L1
ttLb-ABE-L5_R	GAGCCATGGTGCTCCCTCCTCCACCCGAG	Primers for dLbCas12a-D156R-ABE-L1
ttLb-ABE-BB-L6_F	AGGTGGGTCTACCATGGCTCCTAAGAAGAAG	Primers for dLbCas12a-D156R-ABE-L4
ttLb-ABE-BB-L6_R	CACCACCTCCGTTGATGGAGCTCTGGGC	Primers for dLbCas12a-D156R-ABE-L4

ttLb-ABE-L6_F	CTCCATCAACGGAGGTGGTGGGTCGAGTG	Primers for dLbCas12a-D156R-ABE-L4
ttLb-ABE-L6_R	GAGCCATGGTAGACCCACCTCCCCCGCT	Primers for dLbCas12a-D156R-ABE-L4
ttLb-ABE-BB-L7_F	CGGTGAGTTCACCATGGCTCCTAAGAAGAAG	Primers for dLbCas12a-D156R-ABE-L5
ttLb-ABE-BB-L7_R	CAGCCGAGCCGTTGATGGAGCTCTGGGC	Primers for dLbCas12a-D156R-ABE-L5
ttLb-ABE-L7_F	CTCCATCAACGGCTCGGCTGGATCAGCC	Primers for dLbCas12a-D156R-ABE-L5
ttLb-ABE-L7_R	GAGCCATGGTGAACTCACCGCTCCCCGC	Primers for dLbCas12a-D156R-ABE-L5
ttLb-ABE-BB-L8_F	GGGTGAATTCACCATGGCTCCTAAGAAGAAG	Primers for dLbCas12a-D156R-ABE-L6
ttLb-ABE-BB-L8_R	CAGCACTTCCGTTGATGGAGCTCTGGGC	Primers for dLbCas12a-D156R-ABE-L6
ttLb-ABE-L8_F	CTCCATCAACGGAAGTGCTGGCTCTGCTG	Primers for dLbCas12a-D156R-ABE-L6
ttLb-ABE-L8_R	GAGCCATGGTGAATTCACCCGAGCCGGC	Primers for dLbCas12a-D156R-ABE-L6
dMb2CBE-BB_F	CGTAAAGCATAAGCGGCCAGCGGCGACG	Primers for dLbCas12a-D156R-CBE with different linkers
dMb2CBE-BB_R	CCCGTGAATACCAACCTTCCGCTTCTTCTTAGGAGCCATG	Primers for dLbCas12a-D156R-CBE with different linkers
dttLb-IN_F	GGAAGGTTGGTATTCACGGGGTGCCTGC	Primers for dLbCas12a-D156R-CBE with different linkers
dttLb-IN_R	CTGGCCGCTTATGCTTTACGGATGTCTGAGC	Primers for dLbCas12a-D156R-CBE with different linkers
D5-OFFT2-HiTom-F1	ggagtgagtacggtgtgcGGCGTATGGTTAAGATGTAC	Hi-Tom primer for off-target editing analysis
D5-OFFT2-HiTom-R1	gagttggatgctggatggATTACGATACTAGTCGAGGG	Hi-Tom primer for off-target editing analysis

TTTTG08-OT1-HiTom-F1	ggagtgagtacggtgtgcGGACTTAGGAGGATGTTTGT	Hi-Tom primer for off-target editing analysis
TTTTG08-OT1-HiTom-R1	gagttggatgctggatggAATTAGCCTTCTCCCAGTTT	Hi-Tom primer for off-target editing analysis
TTTTG08-OT2-HiTom-F1	ggagtgagtacggtgtgcAGCGGATATAAAAATCCCCA	Hi-Tom primer for off-target editing analysis
TTTTG08-OT2-HiTom-R1	gagttggatgctggatggTATTATCTCGTCTCGAACCT	Hi-Tom primer for off-target editing analysis
ATTC02-OT1-HiTom-F1	ggagtgagtacggtgtgcACGTGCTCCTCGACATGTGA	Hi-Tom primer for off-target editing analysis
ATTC02-OT1-HiTom-R1	gagttggatgctggatggAGAAACCTAGGTGGTTCGCG	Hi-Tom primer for off-target editing analysis
ATTC02-OT2-HiTom-F1	ggagtgagtacggtgtgcGTCGACGAGAACCTCTTCTT	Hi-Tom primer for off-target editing analysis
ATTC02-OT2-HiTom-R1	gagttggatgctggatggCGAACTTGAGCTTGTACAGC	Hi-Tom primer for off-target editing analysis
TTTTG05-OT1-HiTom-F1	ggagtgagtacggtgtgcATGAGCTCTACTGTTTTCCC	Hi-Tom primer for off-target editing analysis
TTTTG05-OT1-HiTom-R1	gagttggatgctggatggTAGACACAAATCACCCTTTG	Hi-Tom primer for off-target editing analysis
ATTTC05-OT1-HiTom-F1	ggagtgagtacggtgtgcTGAGAATGACACATAGGGCA	Hi-Tom primer for off-target editing analysis
ATTTC05-OT1-HiTom-R1	gagttggatgctggatggCACTGCTTACTCCATCTTCA	Hi-Tom primer for off-target editing analysis
ATTTC05-OT2-HiTom-F1	ggagtgagtacggtgtgcATAGGAGTAGTGGCTAGACT	Hi-Tom primer for off-target editing analysis
ATTTC05-OT2-HiTom-R1	gagttggatgctggatggCACAAGACATAGGGAACATT	Hi-Tom primer for off-target editing analysis
ATTTA02-OT1-HiTom-F1	ggagtgagtacggtgtgcCGCTACACTAATCTCGTTGG	Hi-Tom primer for off-target editing analysis
ATTTA02-OT1-HiTom-R1	gagttggatgctggatggAGAAAGACTGAAGCTGATGAC	Hi-Tom primer for off-target editing analysis
CTTTA01-OT1-HiTom-F1	ggagtgagtacggtgtgcCCTCATGCCATGCAATTCAA	Hi-Tom primer for off-target editing analysis
CTTTA01-OT1-HiTom-R1	gagttggatgctggatggAAATCGCCGGTTTGTTACTG	Hi-Tom primer for off-target editing analysis

TTTTC04-OT1-HiTom-F1	ggagtgagtacggtgtgcAATTTGTTGGTGCAAAGTCG	Hi-Tom primer for off-target editing analysis
TTTTC04-OT1-HiTom-R1	gagttggatgctggatggGCCCTATGAGTGTTACCTAC	Hi-Tom primer for off-target editing analysis
TTTTC04-OT2-HiTom-F1	ggagtgagtacggtgtgcAGTCGTATTCAGGTGTGAGG	Hi-Tom primer for off-target editing analysis
TTTTC04-OT2-HiTom-R1	gagttggatgctggatggTTGACCTATACCTGCCAGTC	Hi-Tom primer for off-target editing analysis
CTTTG02-OT1-HiTom-F1	ggagtgagtacggtgtgcGTGATCGTGAGATAGAAGCA	Hi-Tom primer for off-target editing analysis
CTTTG02-OT1-HiTom-R1	gagttggatgctggatggATGCAAGAATACTTTGCTCC	Hi-Tom primer for off-target editing analysis
gB-CBE-Linker5	GATCATCAGGGTTGTCCCTTCCAGCCGTGGGATGGT CTTGATGAGCATTCGCAAGCTTTGAGTGGCAGGCTTC GCGCGATACTTCAGAACCAGGGTaacGGAGGGGGAG GGTCGGGAGGTGGTGGCAGCGGAGGAGGCGGTAGC GGAGGTGGAGGATCAGGTGGCGGAGGGTCGGGAGG GGGCGGTTCTGGTGGTGGGGGCTCGGGTGGAGGAG GGAGCaccatggctccgaagaagaagaggaaggttggcatccacgggg tgccagctgctCTGTTTCAAGATTTTACA	gBlock of (GGGGS)x8 for dCas12a base editor
gB-CBE-Linker6	GATCATCAGGGTTGTCCCTTCCAGCCGTGGGATGGT CTTGATGAGCATTCGCAAGCTTTGAGTGGCAGGCTTC GCGCGATACTTCAGAACCAGGGTaacGGAGGTGGTGG GTCGAGTGGTTCTGAGACGCCAGGGACGTCCGAATC GGCGACGCCGGAAAGCGGGGGAGGTGGGTCTaccatg gctccgaagaagaagaggaaggttggcatccacggggtgccagctgctCT GTTTCAAGATTTTACA	gBlock of GGGS-XTEN-GGGS for dCas12a base editor

gB-CBE-Linker7	GATCATCAGGGTTGTCCCTTCCAGCCGTGGGATGGT CTTGATGAGCATTCGCAAGCTTTGAGTGGCAGGCTTC GCGCGATACTTCAGAACCAGGGTaacGGCTCGGCTGG ATCAGCCGCCGGGTCGGGCGAGTTCGGGAGCGCCG GGTCGGCGGCGGGGAGCGGTGAGTTCaccatggctccga agaagaagaggaaggttggcatccacggggtgccagctgctCTGTTTC AAGATTTTACA	gBlock of (GSAGSAAGSGEF)x2 for dCas12a base editor
gB-CBE-Linker8	GATCATCAGGGTTGTCCCTTCCAGCCGTGGGATGGT CTTGATGAGCATTCGCAAGCTTTGAGTGGCAGGCTTC GCGCGATACTTCAGAACCAGGGTaacGGAAGTGCTGG CTCTGCTGCGGGTTCGGGTGAATTTGGTTCTGCGGG ATCGGCGGCAGGGAGTGGGGAGTTTGGTTCAGCTGG GTCAGCGGCCGGCTCGGGTGAATTCaccatggctccgaaga agaagaggaaggttggcatccacggggtgccagctgctCTGTTTCAAG ATTTTACA	gBlock of (GSAGSAAGSGEF)x3 for dCas12a base editor

Supplemental Table 2. T-DNA constructs used in this study

Construct	Cas12a entry clone	Target sites	Purpose
pLR4184	hA3A-Y130F-dFnCas12a-UGI	ATTTC05	dCas12a based C to T base editor in rice
pLR4185	hA3A-Y130F-dFnCas12a-UGI	AC02 (TTTV/TTV)	dCas12a based C to T base editor in rice
pLR4186	hA3A-Y130F-dMb2Cas12a-UGI	ATTTC05	dCas12a based C to T base editor in rice
pLR4187	hA3A-Y130F-dMb2Cas12a-UGI	AC02 (TTTV/TTV)	dCas12a based C to T base editor in rice
pLR4188	hA3A-Y130F-dLbCas12a-UGI	ATTTC05	dCas12a based C to T base editor in rice
pLR4189	hA3A-Y130F-dLbCas12a-UGI	AC02 (TTTV/TTV)	dCas12a based C to T base editor in rice
pLR4190	hA3A-Y130F-dLbCas12a-D156R-UGI	ATTTC05	dCas12a based C to T base editor in rice
pLR4191	hA3A-Y130F-dLbCas12a-D156R-UGI	AC02 (TTTV/TTV)	dCas12a based C to T base editor in rice
pLR4200	ecTadA8e-dFnCas12a	TTTTC04	dCas12a based A to G base editor in rice
pLR4201	ecTadA8e-dFnCas12a	CG02 (TTTV/TTV)	dCas12a based A to G base editor in rice
pLR4202	ecTadA8e-dMb2Cas12a	TTTTC04	dCas12a based A to G base editor in rice
pLR4203	ecTadA8e-dMb2Cas12a	CG02 (TTTV/TTV)	dCas12a based A to G base editor in rice
pLR4204	ecTadA8e-dLbCas12a	TTTTC04	dCas12a based A to G base editor in rice
pLR4205	ecTadA8e-dLbCas12a	CG02 (TTTV/TTV)	dCas12a based A to G base editor in rice
pLR4206	ecTadA8e-dLbCas12a-D156R	TTTTC04	dCas12a based A to G base editor in rice
pLR4207	ecTadA8e-dLbCas12a-D156R	CG02 (TTTV/TTV)	dCas12a based A to G base editor in rice
pLR4746	hA3A-Y130F-linker1-dLbCas12a-D156R-UGI	ATTTC05, TTTTG05, TTTTG08, AC02 (TTTV/TTV)	dCas12a based C to T base editor in rice
pLR4747	hA3A-Y130F-linker2-dLbCas12a-D156R-UGI		dCas12a based C to T base editor in rice
pLR4748	hA3A-Y130F-linker4-dLbCas12a-D156R-UGI		dCas12a based C to T base editor in rice
pLR4749	hA3A-Y130F-linker5-dLbCas12a-D156R-UGI		dCas12a based C to T base editor in rice
pLR4750	hA3A-Y130F-linker6-dLbCas12a-D156R-UGI		dCas12a based C to T base editor in rice
pLR4751	hA3A-Y130F-linker7-dLbCas12a-D156R-UGI		dCas12a based C to T base editor in rice
pLR4752	hA3A-Y130F-linker8-dLbCas12a-D156R-UGI		dCas12a based C to T base editor in rice

pLR4753	ecTadA8e-linker3-dLbCas12a-D156R	TTTTC04, CG02 (TTTV/TTV), AA02 (TTTV/TTV), CA01 (TTTV/TTV)	dCas12a based A to G base editor in rice
pLR4754	ecTadA8e-linker4-dLbCas12a-D156R		dCas12a based A to G base editor in rice
pLR4755	ecTadA8e-linker5-dLbCas12a-D156R		dCas12a based A to G base editor in rice
pLR4756	ecTadA8e-linker6-dLbCas12a-D156R		dCas12a based A to G base editor in rice
pLR4757	ecTadA8e-linker7-dLbCas12a-D156R		dCas12a based A to G base editor in rice
pLR4758	ecTadA8e-linker8-dLbCas12a-D156R		dCas12a based A to G base editor in rice

Supplemental Table 3. crRNAs used in this study

Targeted gene	Targeted site	Spacer sequence with PAM (5'- 3')*
LOC_Os02g45250	ATTTC05	**TTTC**TGCTTCCTGCAATGCCGGTAGAC
LOC_Os03g48170	ATTTC02	**TTTC**GTCTCCCTGCAAACCACCGCGCA
LOC_Os03g08570	TTTTG05	**TTTG**TTCAAAACCCTTAGAGATATCTA
Osa-miR528	TTTTG08	**TTTG**CCTCTCTCTCCTGTGCTTGCCTC
LOC_Os09g26999	TTTTC04	**TTTC**CAGAAAGAGAAGGAGGCACAGAT
LOC_Os07g40404	CTTTG02	**TTTG**GCACCATATGCTTGCTGATCAAA
LOC_Os02g43194	ATTTA02	**TTTA**CCGGTGAAAAGGACCTTGTCCCA
LOC_Os07g10860	CTTTA01	**TTTA**TACGTGGAAACAATGACAGTTCA
LOC_Os06g22970	ATTC02	**ATTC**GTCTCCCTGCAAACCACCGCGCA
LOC_Os07g40790	CTTG02	**CTTG**GCACCATATGCTTGCTGATCAAA
LOC_Os04g45720	ATTA02	**ATTA**CCGGTGAAAAGGACCTTGTCCCA
LOC_Os07g47284	CTTA01	**CTTA**TACGTGGAAACAATGACAGTTCA

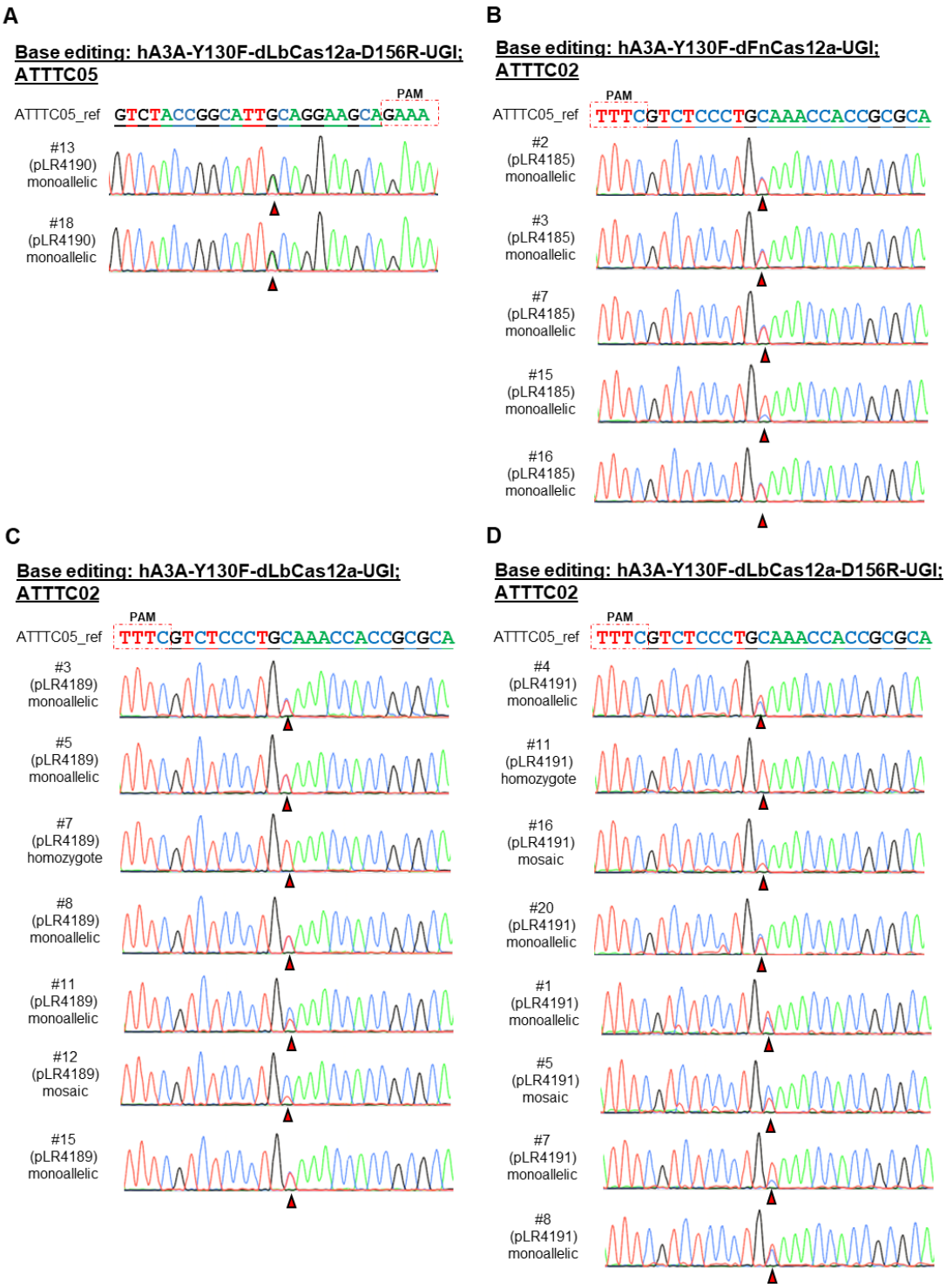
* **The PAM is in bold.**

Supplemental Table 4. Potential off-target sites

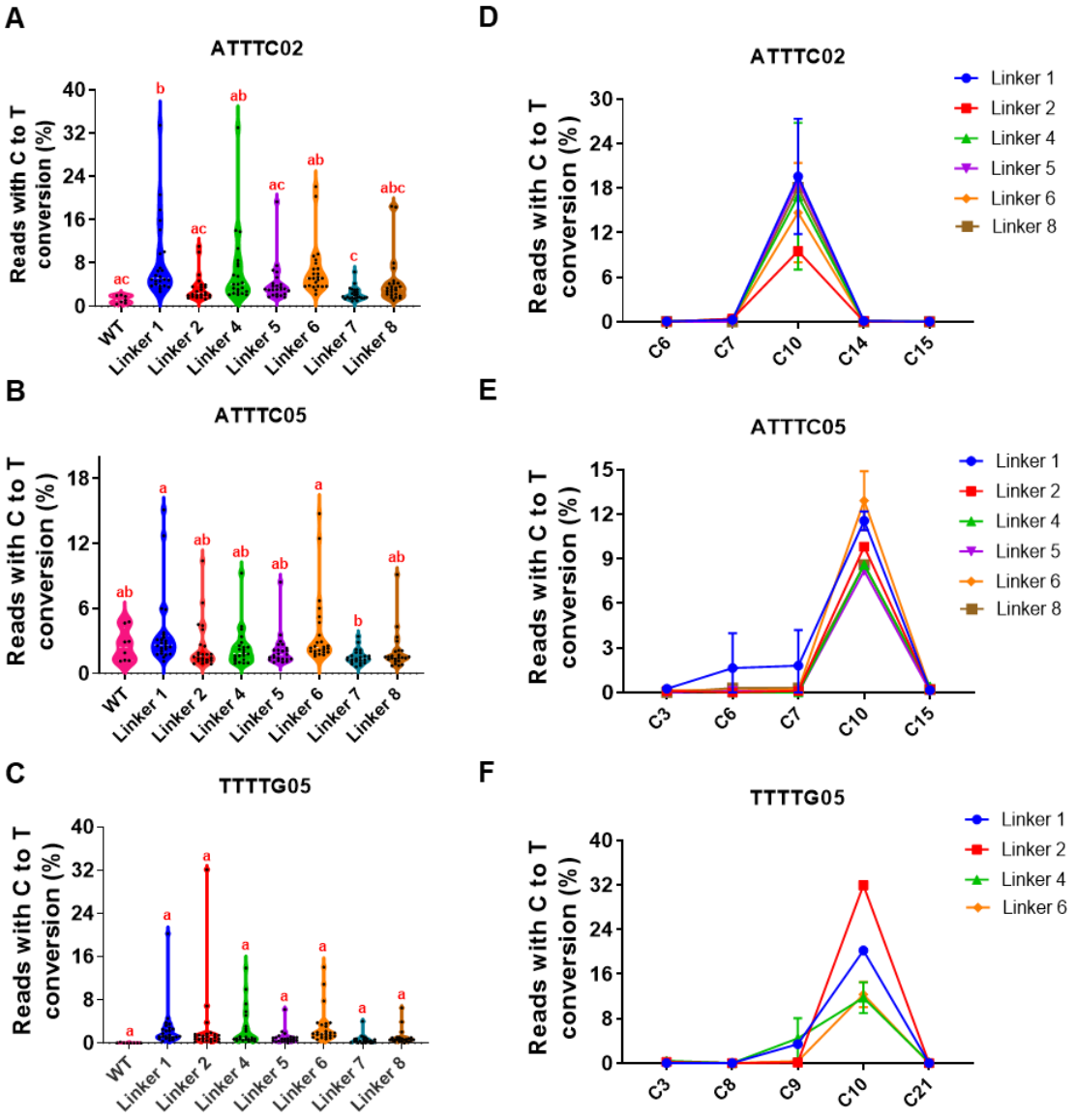
Targeted gene	Targeted site	Spacer sequence with PAM (5'- 3')*	No. of mismatches
LOC_Os09g26999	TTTTC04	**TTTC**CAGAAAGAGAAGGAGGCACAGAT	On target
/	TTTTC04-OT1	**ATTC**CAGAAAcAaAAGGAaaCACAGAT	4
LOC_Os05g09360	TTTTC04-OT2	**ATTT**aAGAAAGAaAAaGAGGCtCAGAT	4
LOC_Os07g40404	CTTTG02	**TTTG**GCACCATATGCTTGCTGATCAAA	On target
LOC_Os06g13060	CTTTG02-OT1	**CTTT**tCACCATATGCTTGCTGtaCAtA	4
LOC_Os07g10860	CTTTA01	**TTTA**TACGTGGAAACAATGACAGTTCA	On target
LOC_Os08g41010	CTTTA01-OT1	**TTTT**gAgGaGaAAACAATGACAGTTCA	4
LOC_Os02g43194	ATTTA02	**TTTA**CCGGTGAAAAGGACCTTGTCCCA	On target
LOC_Os08g02560	ATTTA02-OT1	**ATTA**CCGGTcAAcAtGAaCTTGTCCCA	4
Osa-miR528	TTTTG08	**TTTG**CCTCTCTCTCCTGTGCTTGCCTC	On target
LOC_Os05g19280	TTTTG08-OT1	**TTTC**aCTCTaTCTtCaGTGtTTGCCTC	5
LOC_Os12g42550	TTTTG08-OT2	**GTTT**CCTCTCTCTCCTcgtCTcGtCTC	5
LOC_Os02g45250	ATTTC05	**TTTC**TGCTTCCTGCAATGCCGGTAGAC	On target
LOC_Os02g52960	ATTTC05-OT1	**ATTA**TGCTTCCTGCAATGttttTtGAC	5
LOC_Os03g11260	ATTTC05-OT2	**CTTA**TGCTTCCaGCtATGgCGtTtGAC	5
LOC_Os03g48170	ATTTC02	**TTTC**GTCTCCCTGCAAACCACCGCGCA	On target
LOC_Os05g28290	ATTTC02-OT1	**CTTC**GcCTtCCTGCAAACaAtCGCGCg	5
LOC_Os01g61160	ATTTC02-OT2	**CTTC**GTCTTCCCGCAGACCACCTCGCT	5
LOC_Os03g08570	TTTTG05	**TTTG**TTCAAAACCCTTAGAGATATCTA	On target
LOC_Os07g39740	TTTTG05-OT1	**TTTT**TTCAAAACgCTcAGtGATATCac	5

* **The PAM is in bold.**

SUPPLEMENTAL FIGURES



Supplemental Figure 1. Representative genotypes of dCas12a-based CBE edits in transgenic rice plants. The edited positions are indicated by red triangles.

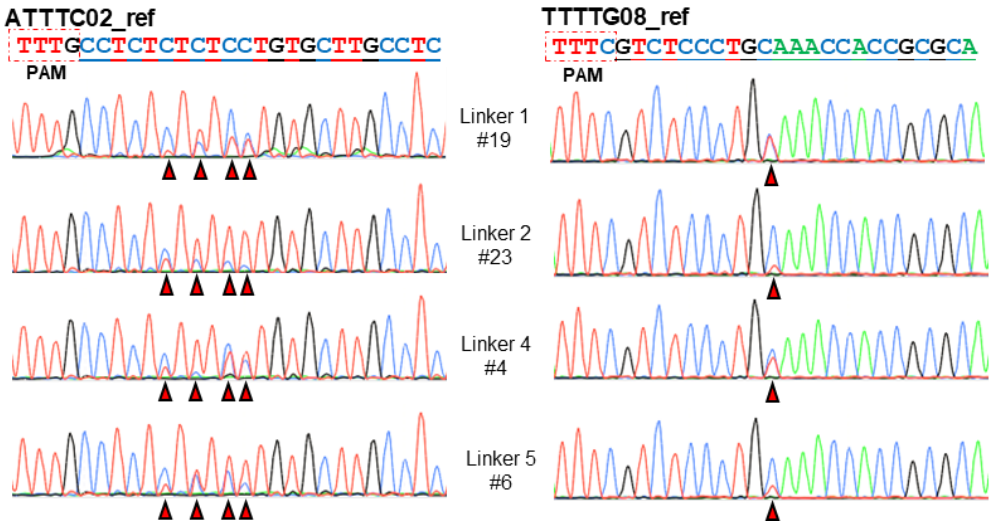


Supplemental Figure 2. Assessment of dLbCas12a-D156R-based CBEs with different linkers for base editing in transgenic rice calli. (**A, D**) The C-to-T editing frequency (A) and editing window (D) at target ATTTC02. (**B, E**) The C-to-T editing frequency (B) and editing window (E) at target ATTTC05. (**C, F**) The C-to-T editing frequency (C) and editing window (F) at target TTTTG05. The editing efficiency was measured by NGS of PCR amplicons. Each dot represents independent callus in **A**, **B** and **C**. The first quartile, median, and third quartile are shown as doted lines. WT, transgenic calli with LbCas12a expressed. Data are presented as mean values ± SD of 22~24 transgenic calli in **D**, **E** and **F**. Different letters indicate significant differences ($P<0.05$; one-way ANOVA, Tukey's test).

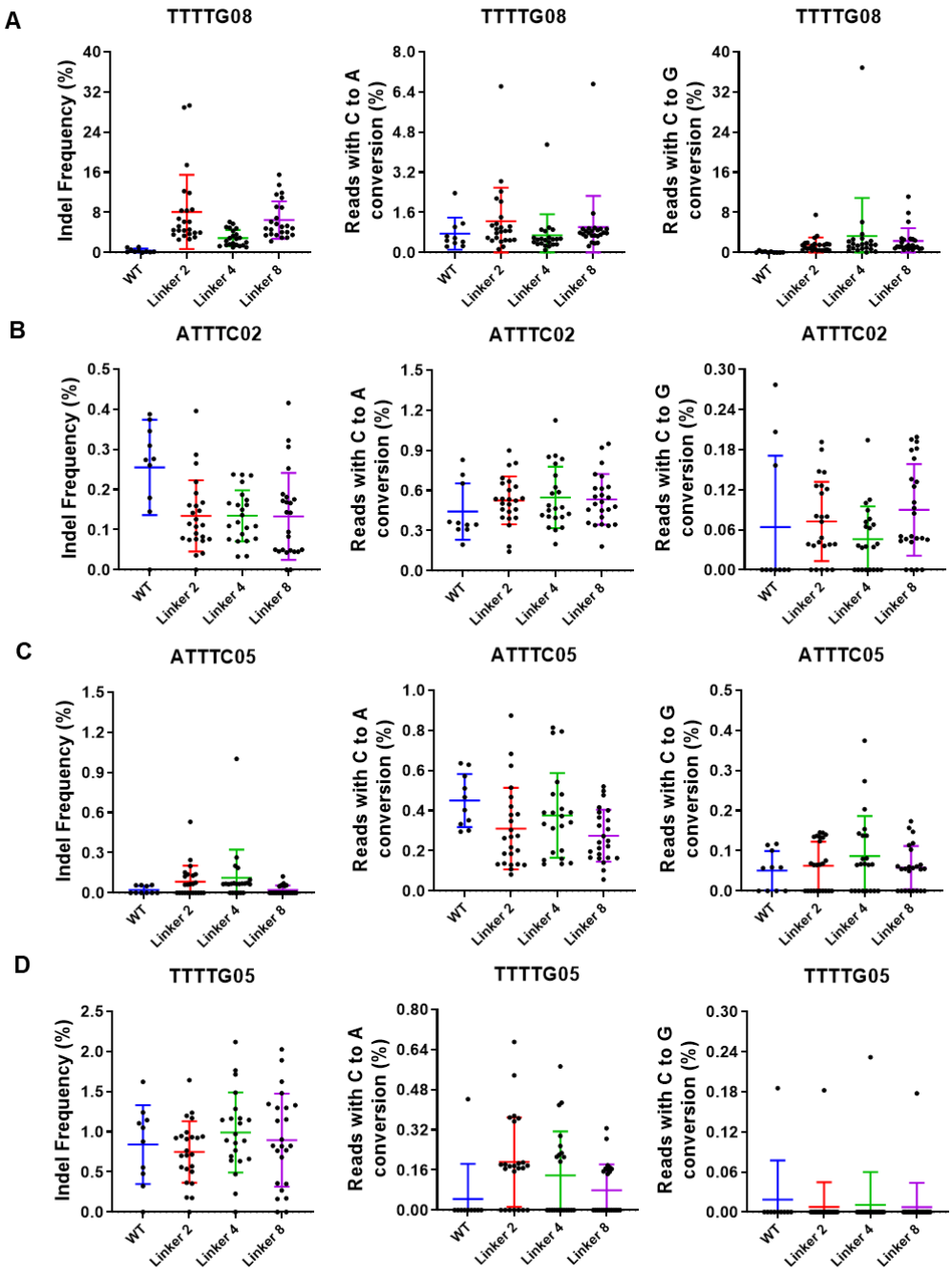
A

Target	Linker	Tested T0 line	Edited T0 lines		Monoallelic Edit		Biallelic Edit	
			number	ratio (%)	number	ratio (%)	number	ratio (%)
TTTTG08	Linker 1	24	24	100	16	66.67	1	4.17
	Linker 2	24	24	100	14	58.33	4	16.67
	Linker 4	22	22	100	13	59.09	6	27.27
	Linker 5	24	24	100	16	66.67	0	0
	Linker 6	23	23	100	20	86.96	0	0
	Linker 7	24	23	95.83	9	37.5	1	4.17
	Linker 8	24	24	100	18	75	3	12.5
ATTTC02	Linker 1	24	6	25	1	4.17	0	0
	Linker 2	24	2	8.33	0	0	0	0
	Linker 4	22	4	18.18	1	4.55	0	0
	Linker 5	24	1	4.17	0	0	0	0
	Linker 6	23	2	8.7	0	0	0	0
	Linker 7	24	0	0	0	0	0	0
	Linker 8	24	2	8.33	0	0	0	0

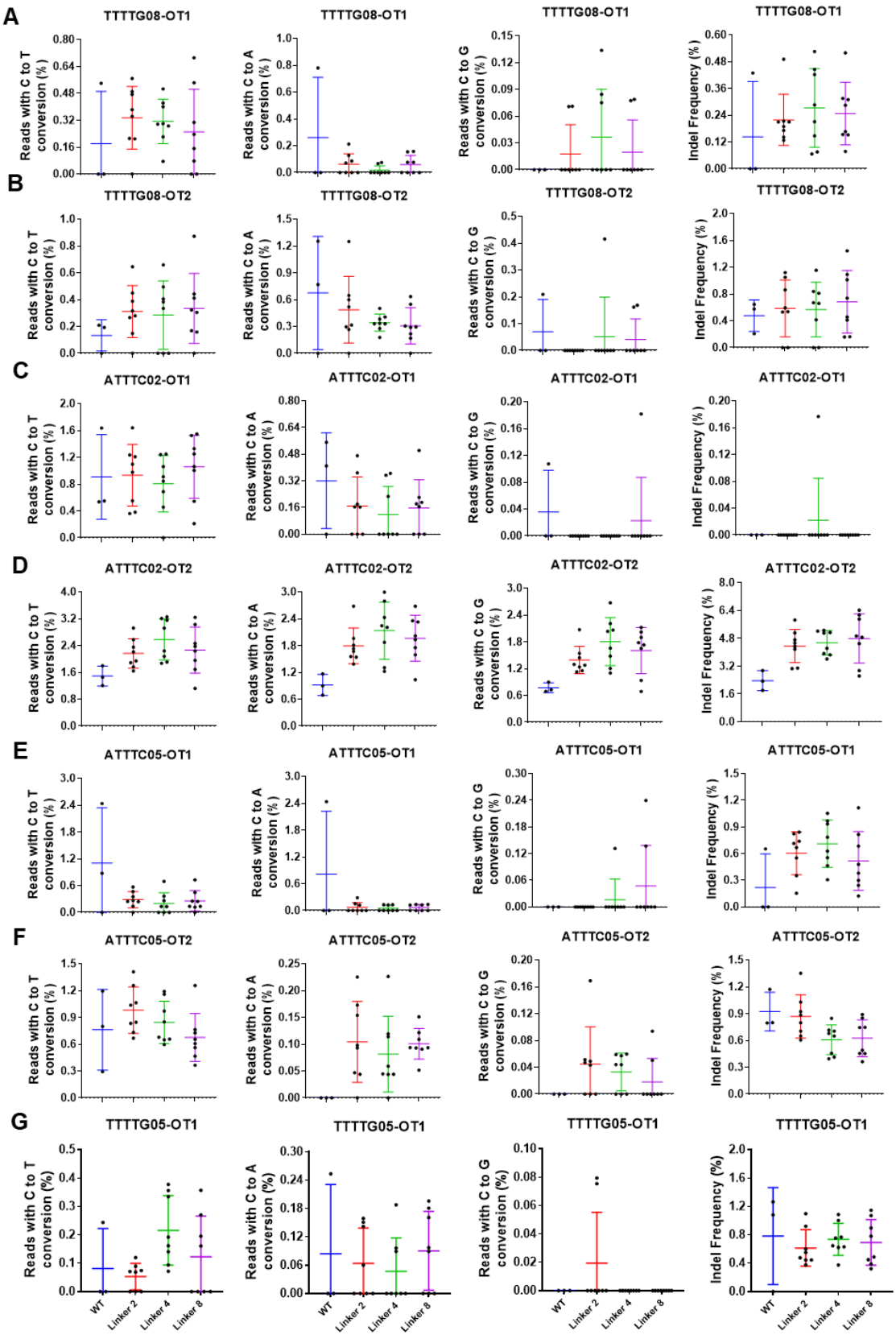
B



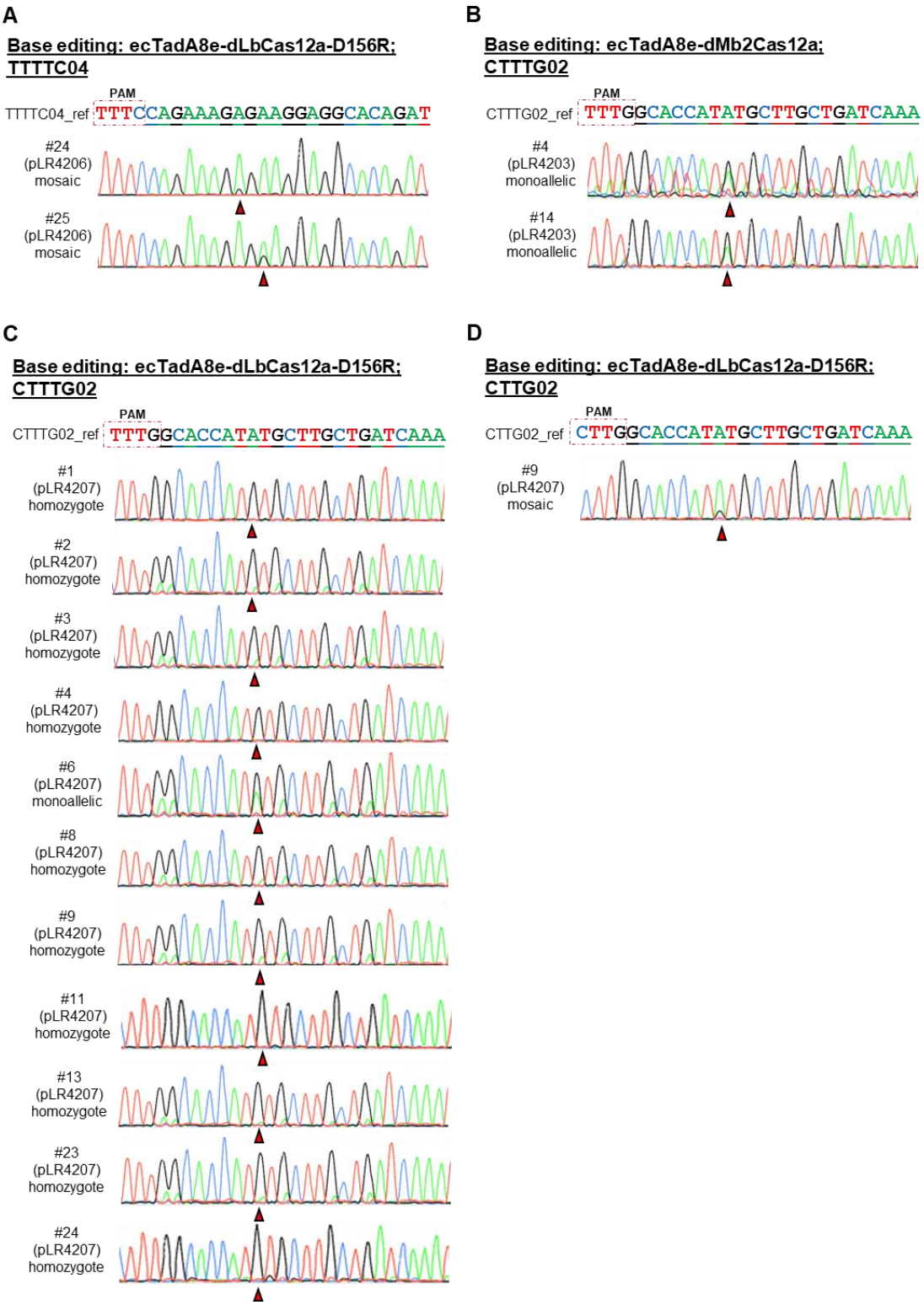
Supplemental Figure 3. The C-to-T base editing profile of dLbCas12a-D156R based CBEs at TTTTG08 and ATTTC02 sites in transgenic rice calli. (A) Genotypes of transgenic rice calli edited by dLbCas12a-D156R based CBEs with different linkers. The calli with C-to-T mutation frequency higher than 10% were regarded as edited. The calli with C-to-T mutation frequency from 30% to 75% were regarded as monoallelic edits. The calli with C-to-T mutation frequency higher than 75% were regarded as biallelic edits. **(B)** Representative genotypes of base-edited rice calli at TTTTG08 and ATTTC02 sites. The edited positions are indicated by red triangles.



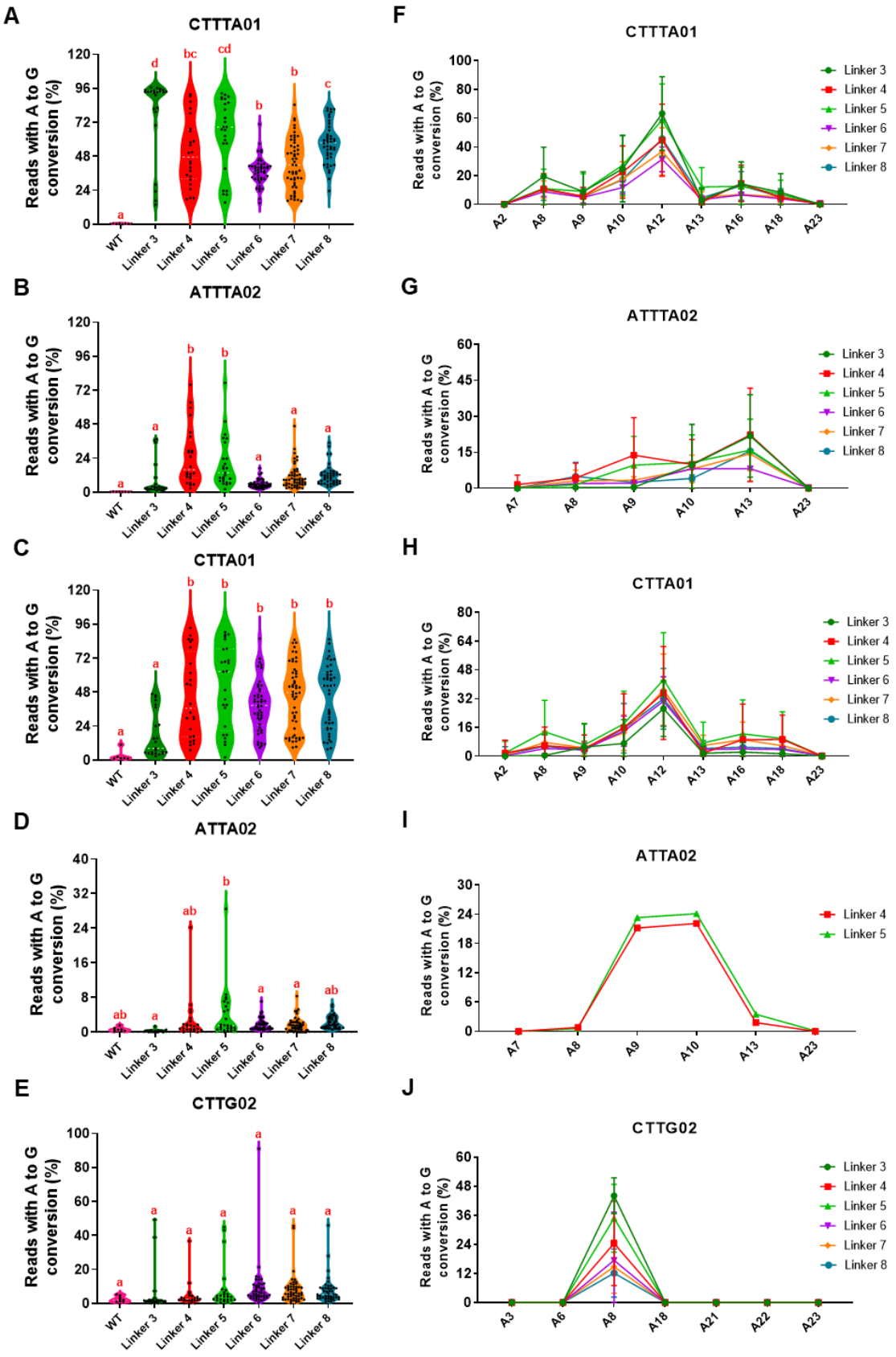
Supplemental Figure 4. Byproducts analysis of dLbCas12a-D156R-based CBEs with different linkers in transgenic rice calli. (A) The indel frequency, C to A editing frequency, and C to G editing frequency at site TTTTG08**. (B)** The indel frequency, C to A editing frequency, and C to G editing frequency at site ATTTC02**. (C)** The indel frequency, C to A editing frequency, and C to G editing frequency at site ATTTC05**. (D)** The indel frequency, C to A editing frequency, and C to G editing frequency at site TTTTG05**.** The editing efficiency was measured by NGS of PCR amplicons. WT, transgenic calli with LbCas12a expressed. Each dot represents independent callus. Data are presented as mean values ± SD of 22~24 transgenic calli.


A
TTTTG08-OT1
Reads with C to T conversion (%)
Reads with C to A conversion (%)
Reads with C to G conversion (%)
Indel Frequency (%)
B
TTTTG08-OT2
C
ATTTC02-OT1
D
ATTTC02-OT2
E
ATTTC05-OT1
F
ATTTC05-OT2
G
TTTTG05-OT1
WT
Linker 2
Linker 4
Linker 8

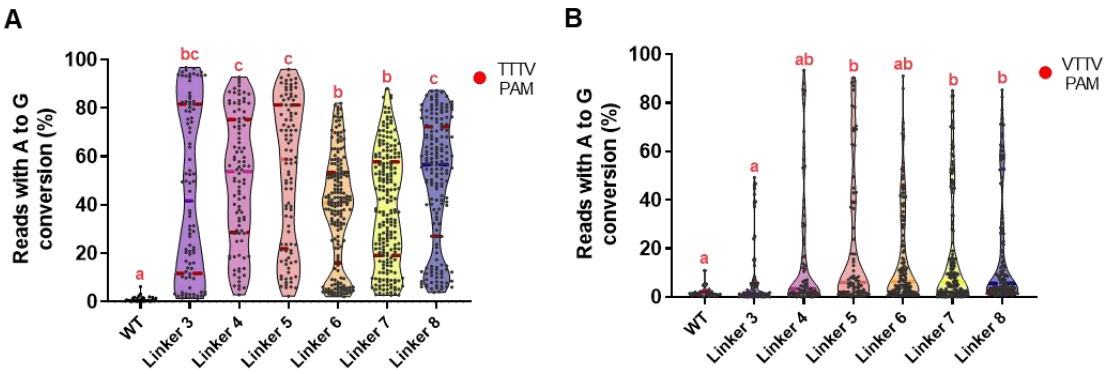
Supplemental Figure 5. Off-target analysis of dLbCas12a-D156R-based CBEs with different linkers in transgenic rice calli. (A) The C to T editing frequency, C to A editing frequency, C to G editing frequency, and indel frequency at off-target site TTTTG08-OT1**. (B)** The C to T editing frequency, C to A editing frequency, C to G editing frequency, and indel frequency at off-target site TTTTG08-OT2**. (C)** The C to T editing frequency, C to A editing frequency, C to G editing frequency, and indel frequency at off-target site ATTTC02-OT1**. (D)** The C to T editing frequency, C to A editing frequency, C to G editing frequency, and indel frequency at off-target site ATTTC02-OT2**. (E)** The C to T editing frequency, C to A editing frequency, C to G editing frequency, and indel frequency at off-target site ATTTC05-OT1**. (F)** The C to T editing frequency, C to A editing frequency, C to G editing frequency, and indel frequency at off-target site ATTTC05-OT2**. (G)** The C to T editing frequency, C to A editing frequency, C to G editing frequency, and indel frequency at off-target site TTTTG05-OT1**.** WT, transgenic calli with LbCas12a expressed. Each dot represents independent callus. Data are presented as mean values ± SD of 22~24 transgenic calli.



Supplemental Figure 6. Representative genotypes of dCas12a-based ABE edits in T0 rice plants. The edited positions are indicated by red triangles.


A
CTTTA01
Reads with A to G conversion (%)
WT
Linker 3
Linker 4
Linker 5
Linker 6
Linker 7
Linker 8
B
ATTTA02
C
CTTA01
D
ATTA02
E
CTTG02
F
CTTTA01
A2
A8
A9
A10
A12
A13
A16
A18
A23
G
ATTTA02
A7
H
CTTA01
I
ATTA02
J
CTTG02
A3
A6
A21
A22

Supplemental Figure 7. The A-to-G editing frequency and editing window analysis of dLbCas12a-D156R-based ABEs at TTTV and VTTV PAM sites in T0 rice plants. (**A, F**) The A-to-G editing frequency (A) and editing window (F) at target CTTTA01. (**B, G**) The A-to-G editing frequency (B) and editing window (G) at target ATTTA02. (**C, H**) The A-to-G editing frequency (C) and editing window (H) at target CTTA01. (**D, I**) The A-to-G editing frequency (D) and editing window (H) at target ATTA02. (**E, J**) The A-to-G editing frequency (E) and editing window (J) at target CTTG02. The editing efficiency was measured by NGS of PCR amplicons. Each dot represents an independent T0 line. The first quartile, median, and third quartile are shown as doted lines. WT, T0 lines with LbCas12a expressed. Data are presented as mean values ± SD of 23~54 independent T0 lines. Different letters indicate significant differences ($P<0.05$; one-way ANOVA, Tukey's test).



Supplemental Figure 8. Assessment of six dLbCas12-D156R-based ABEs with different linkers for base editing in T0 rice plants. (**A**) The A-to-G editing frequency at four sites with TTTV PAMs. (**B**) The A-to-G editing frequency at three sites with VTTV PAMs. Each dot represents an independent T0 line. The first quartile, median, and third quartile are shown as dash lines. The editing efficiency was measured by NGS of PCR amplicons. Different letters indicate significant differences ($P<0.05$; one-way ANOVA, Tukey test). WT, T0 lines with LbCas12a expressed.

A

Linker 5 #19 (pLR4755 T0 line)

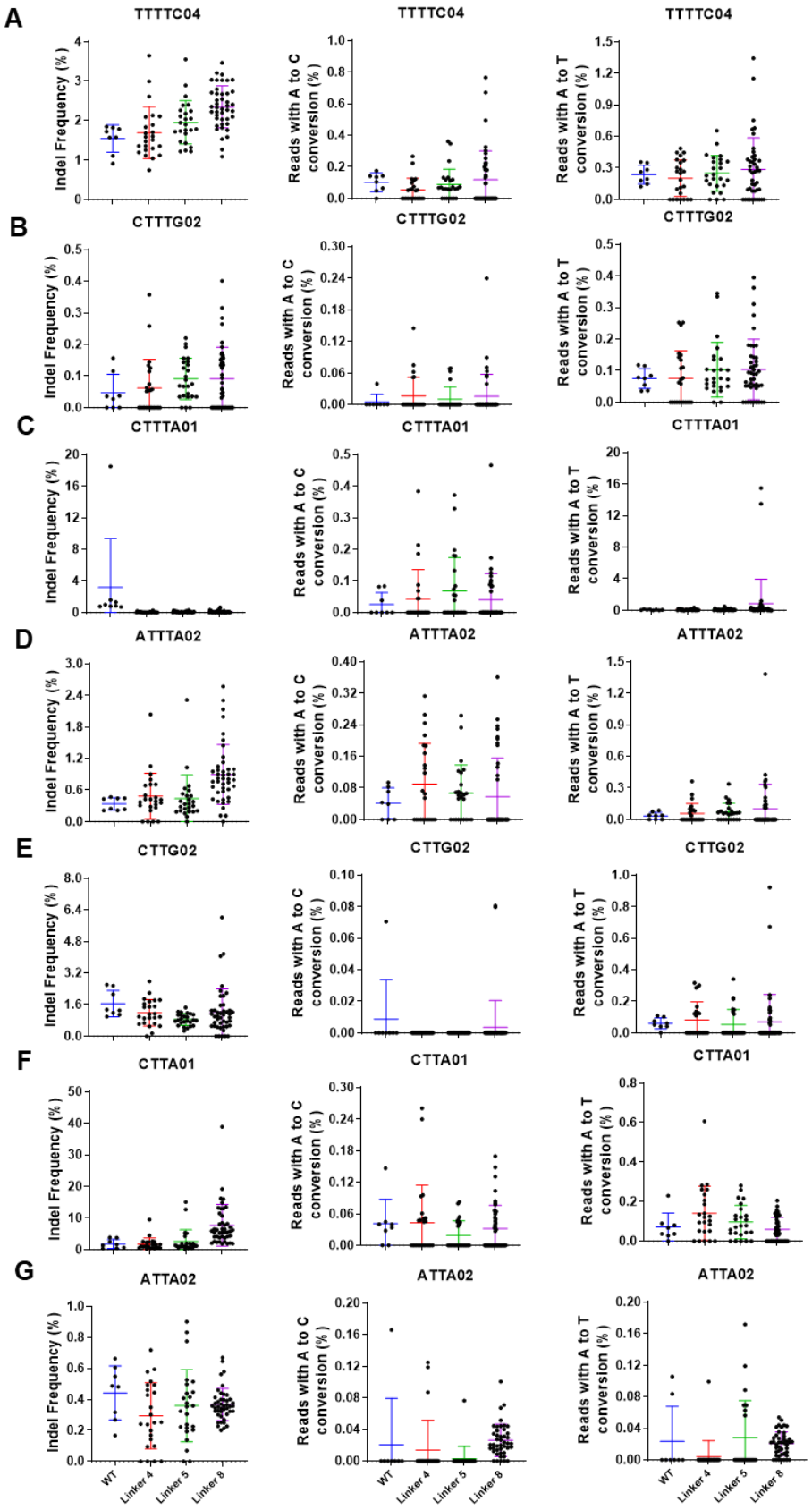
		T	T	T	C	C	A2	G	A4	A5	A6	G	A8	G	A10	A11	G	G	A14	G	G	C	A18	C	A20	G	A22	T
TTTTC04	A	0	0	0	0	0	99.9	0	99.9	100	100	0	93.7	0	13.5	58.1	0	0	99.5	0	0.3	0.1	99.9	0	99.8	0	100	0
	G	0	0	0	0	0	0.1	100	0	0	0	99.9	6.3	99.9	86.5	41.8	99.8	99.9	0.5	100	99.7	0	0.1	0	0.2	100	0	0
		T	T	T	A	T	A2	C	G	T	G	G	A8	A9	A10	C	A12	A13	T	G	A16	C	A18	G	T	T	C	A23
CTTTA01	A	0	0	0	100	0	99.9	0	0	0	0	0	98.5	98.8	15.4	0	10.3	97.1	0	0	98.1	0	57.7	0	0	0	0	100
	G	0	0	0	0	0	0.1	0	100	0	100	100	1.5	1.2	84.6	0	89.7	2.9	0	100	1.9	0	42.3	100	0	0	0	0
		T	T	T	G	G	C	A3	C	C	A6	T	A8	T	G	C	T	T	G	C	T	G	A18	T	C	A21	A22	A23
CTTTG02	A	0.1	0.1	0	0	0.5	0	99.9	0	0	99.9	0	25	0	0.2	0	0	0	0	0	0	0.3	100	0	0	99.8	100	100
	G	0	0	0	100	99.5	0	0.1	0	0	0.1	0	75	0	99.7	0	0	0	100	0	0	99.7	0	0	0	0.2	0	0
		T	T	T	A	C	C	G	G	T	G	A7	A8	A9	A10	G	G	A13	C	C	T	T	G	T	C	C	C	A23
ATTTA02	A	0	0	0	100	0	0	0.1	0.3	0	0	100	99.9	98.6	68.2	0	0	97.6	0	0	0	0	0	0	0	0	0	100
	G	0	0	0	0	0.1	0	99.5	99.7	0	100	0	0.1	1.3	31.8	100	100	2.4	0	0	0	0	99.9	0	0	0	0	0
		C	T	T	A	T	A2	C	G	T	G	G	A8	A9	A10	C	A12	A13	T	G	A16	C	A18	G	T	T	C	A23
CTTA01	A	0	0	0	100	0	100	0.1	0.2	0	0	0	97.8	98.5	59.6	0	11.5	99.3	0	0	97	0	97.8	0	0	0	0.1	100
	G	0	0	0	0	0	0	0	99.8	0	100	100	2.2	1.5	40.4	0	88.5	0.7	0	100	3	0	2.2	100	0	0	0	0
		C	T	T	G	G	C	A3	C	C	A6	T	A8	T	G	C	T	T	G	C	T	G	A18	T	C	A21	A22	A23
CTTG02	A	0	0	0	0	0	0	100	0	0	100	0	96.9	0	0	0	0	0	0	0	0	0.2	100	0	0	99.8	100	100
	G	0	0	0	100	100	0	0	0	0	0	0	3.1	0	100	0	0	0	100	0	0	99.8	0	0	0	0.2	0	0
		A	T	T	A	C	C	G	G	T	G	A7	A8	A9	A10	G	G	A13	C	C	T	T	G	T	C	C	C	A23
ATTA02	A	99.9	0	0	99.8	0	0.1	0	0.2	0.1	0	100	99.9	99.9	99.6	0	0	99.9	0	0.1	0	0	0	0.1	0	0.1	0	100
	G	0	0	0	0.2	0	0	100	99.8	0	100	0	0	0.1	0.1	99.9	100	0.1	0	0	0	0	100	0	0	0	0	0

B

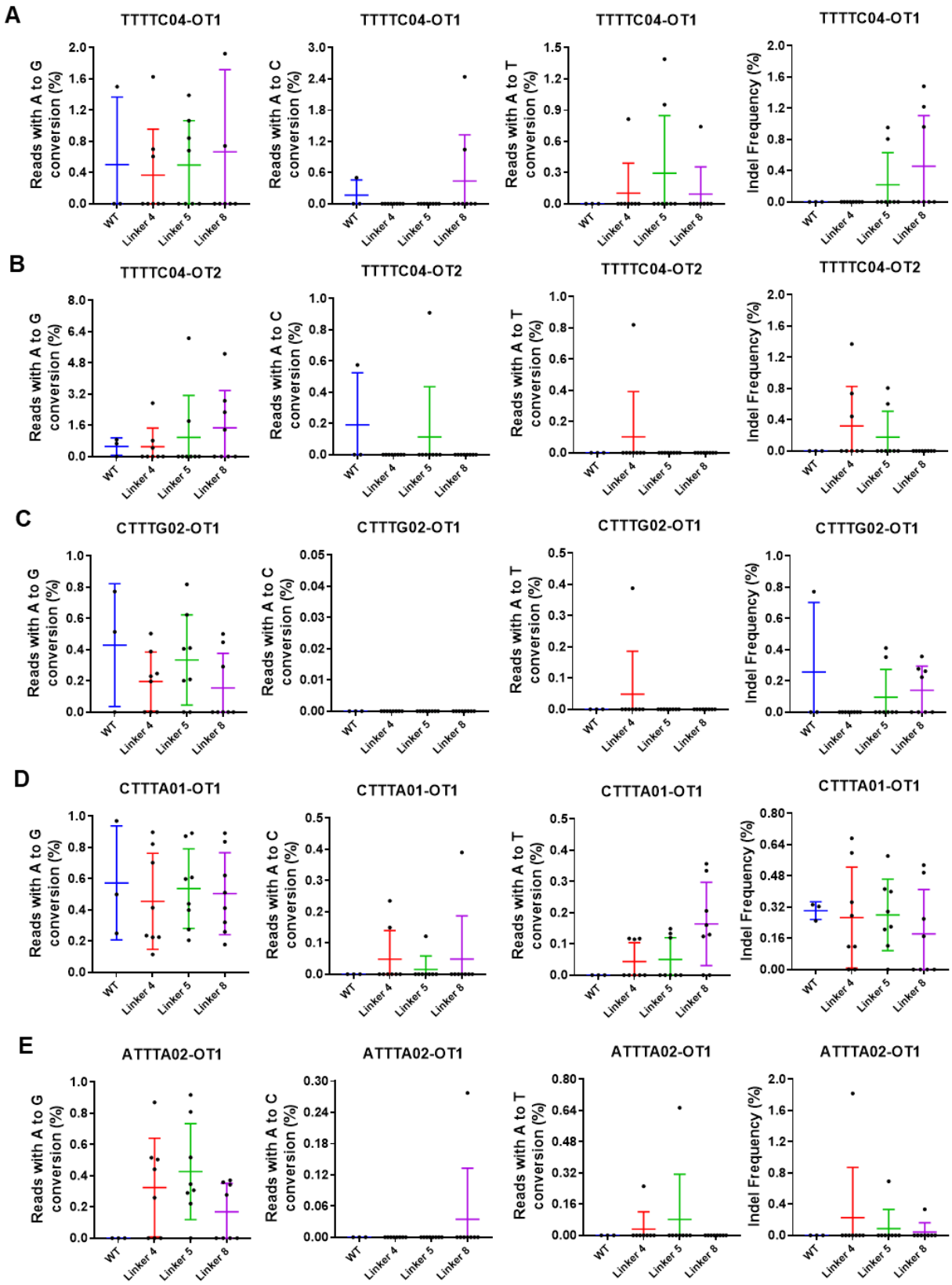
Linker 5 #23 (pLR4755 T0 line)

		T	T	T	C	C	A2	G	A4	A5	A6	G	A8	G	A10	A11	G	G	A14	G	G	C	A18	C	A20	G	A22	T
TTTTC04	A	0	0	0	0.1	0	99.7	0	100	100	99.9	0	56.7	0	10.9	91.4	0	0	99.4	0	0.5	0	99.6	0	99.8	0	100	0
	G	0	0	0	0	0	0.2	100	0	0	0	100	43.3	100	89.1	8.6	99.9	100	0.6	100	99.5	0	0.4	0	0	100	0	0
		T	T	T	A	T	A2	C	G	T	G	G	A8	A9	A10	C	A12	A13	T	G	A16	C	A18	G	T	T	C	A23
CTTTA01	A	0	0	0	100	0	99.9	0	0.4	0	0	0	95.3	98.1	89.2	0	13.1	60.7	0	0	96.2	0	97.7	0	0	0	0	99.7
	G	0	0.1	0	0	0	0.1	0	99.6	0	100	100	4.7	1.9	10.8	0	86.9	39.3	0	100	3.8	0	2.3	100	0	0	0	0.3
		T	T	T	G	G	C	A3	C	C	A6	T	A8	T	G	C	T	T	G	C	T	G	A18	T	C	A21	A22	A23
CTTTG02	A	0	0	0	0	0.2	0	100	0	0	100	0	16.2	0	0	0.1	0	0	0.1	0	0	0	100	0	0	100	100	100
	G	0	0	0	100	99.8	0.1	0	0	0	0	0	83.8	0	100	0	0	0	99.9	0	0	100	0	0	0	0	0	0
		T	T	T	A	C	C	G	G	T	G	A7	A8	A9	A10	G	G	A13	C	C	T	T	G	T	C	C	C	A23
ATTTA02	A	0	0	0	100	0	0.1	0.3	0.1	0	0	100	99.6	95.9	94.9	0	0	58.3	0	0	0	0	0	0	0	0	0.1	100
	G	0	0	0	0	0	0	99.3	99.7	0	100	0	0.4	4.1	5.1	99.9	100	41.7	0	0	0	0	100	0	0	0	0	0
		C	T	T	A	T	A2	C	G	T	G	G	A8	A9	A10	C	A12	A13	T	G	A16	C	A18	G	T	T	C	A23
CTTA01	A	0	0	0	100	0	98.7	0	0.2	0	0	0	95.3	64.3	59.7	0	15.8	98.4	0	0	95.3	0	96.5	0.1	0	0	0	100
	G	0	0	0	0	0	1.3	0	99.8	0	100	100	4.7	35.7	40.3	0	84.2	1.6	0	100	4.7	0	3.5	99.9	0	0	0	0
		C	T	T	G	G	C	A3	C	C	A6	T	A8	T	G	C	T	T	G	C	T	G	A18	T	C	A21	A22	A23
CTTG02	A	0	0	0	0	0	0	100	0	0	100	0	55	0	0	0	0	0	0	0	0	0	100	0	0	100	100	100
	G	0	0	0	100	100	0	0	0	0	0	0	45	0	100	0	0	0	100	0	0	100	0	0	0	0	0	0
		A	T	T	A	C	C	G	G	T	G	A7	A8	A9	A10	G	G	A13	C	C	T	T	G	T	C	C	C	A23
ATTA02	A	99.9	0	0	100	0	0	0	0.2	0	0	100	99.9	99.8	99.9	0	0	99.8	0.1	0	0	0	0	0	0	0	0	99.9
	G	0	0	0	0	0	0	99.8	99.8	0	100	0	0	0.2	0.1	100	100	0.2	0	0	0	0	100	0	0	0	0	0

Supplemental Figure 9. Representative genotypes of seven target sites in dLbCas12a-D156R-Linker 5-ABE in T0 rice plants. Multiplexed A-to-G base editing of seven target sites in rice T0 lines #19 (**A**) and #23 (**B**) by dLbCas12a-D156R-Linker 5-ABE. The DNA bases Adenine [A] with the number indicating the position in the protospacer are highlighted in red, and the other DNA bases of the protospacer are highlighted in green. The PAM is highlighted in yellow. The numbers indicate the percentages of A or G in total sequencing reads obtained by NGS of PCR amplicons.


A
TTTTC04
Indel Frequency (%)
Reads with A to C conversion (%)
Reads with A to T conversion (%)
B
CTTTG02
C
CTTTA01
D
ATTTA02
E
CTTG02
F
CTTA01
G
ATTA02
WT
Linker 4
Linker 5
Linker 8

Supplemental Figure 10. Byproducts analysis of dLbCas12a-D156R-based ABEs with different linkers in T0 rice plants. (A) The indel frequency, A to C editing frequency, and A to T editing frequency at site TTTTC04**. (B)** The indel frequency, A to C editing frequency, and A to T editing frequency at site CTTTG02**. (C)** The indel frequency, A to C editing frequency, and A to T editing frequency at site CTTTA01**. (D)** The indel frequency, A to C editing frequency, and A to T editing frequency at site ATTTA02**. (E)** The indel frequency, A to C editing frequency, and A to T editing frequency at site CTTG02**. (F)** The indel frequency, A to C editing frequency, and A to T editing frequency at site CTTA01**. (G)** The indel frequency, A to C editing frequency, and A to T editing frequency at site ATTA02**.** The editing efficiency was measured by NGS of PCR amplicons. WT, rice T0 plants with LbCas12a expressed. Each dot represents independent line. Data are presented as mean values ± SD.


A
TTTTC04-OT1
Reads with A to G conversion (%)
Reads with A to C conversion (%)
Reads with A to T conversion (%)
Indel Frequency (%)
B
TTTTC04-OT2
C
CTTTG02-OT1
D
CTTTA01-OT1
E
ATTTA02-OT1
WT
Linker 4
Linker 5
Linker 8

Supplemental Figure 11. Off-target analysis of dLbCas12a-D156R-based ABEs with different linkers in T0 rice plants. (A) The A to G editing frequency, A to C editing frequency, A to T editing frequency, and indel frequency at off-target site TTTTC04-OT1**. (B)** The A to G editing frequency, A to C editing frequency, A to T editing frequency, and indel frequency at off-target site TTTTC04-OT2**. (C)** The A to G editing frequency, A to C editing frequency, A to T editing frequency, and indel frequency at off-target site CTTTG02-OT1**. (D)** The A to G editing frequency, A to C editing frequency, A to T editing frequency, and indel frequency at off-target site CTTTA01-OT1**. (E)** The A to G editing frequency, A to C editing frequency, A to T editing frequency, and indel frequency at off-target site ATTTA02-OT1**.** WT, rice T0 plants with LbCas12a expressed. Each dot represents independent line. Data are presented as mean values ± SD.