SUPPLEMENTAL MATERIAL for "A Versatile and Efficient Plant Protoplast Platform for Genome Editing by Cas9 RNPs"

RNP Exp. 2 89.5 %

*AtBON*1 gRNA1 *AtBON*1 gRNA2

```
TCCGGTGCTGGTGCTACAGCCGGCGTTGGTGGAAGTGGTTCCTCCGCCGCTCTCGGTGCGACTAACGACGCCCTTG  WT
TCCGGTGCTGGTGCTACAGCCGG------------------------------TCGGTGCGACTAACGACGCCCTTG  87.5%
TCCGGTGCTGGTGCTACAGCCGGCGTTGGTGGAAGTGGTTCCTCCGCCGCTCTCGGTGCGACTAACGACGCCCTTG  10.3 %
TCCGGTGCTGGTGCTACAGCCGGCG--------------------------------GTGCGACTAACGACGCCCTTG  2.0 %
```

RNP Exp. 3 86.7 %

*AtBON*1 gRNA1 *AtBON*1 gRNA2

```
TCCGGTGCTGGTGCTACAGCCGGCGTTGGTGGAAGTGGTTCCTCCGCCGCTCTCGGTGCGACTAACGACGCCCTTG  WT
TCCGGTGCTGGTGCTACAGCCGG------------------------------TCGGTGCGACTAACGACGCCCTTG  70.4 %
TCCGGTGCTGGTGCTACAGCCGGCGTTGGTGGAAGTGGTTCCTCCGCCGCTCTCGGTGCGACTAACGACGCCCTTG  13.3 %
TCCGGTGCTGGTGCTACAGCCG------------------------------TCGGTGCGACTAACGACGCCCTTG  3.5 %
TCCGGTGCTGGTGCTACAGCCGGCG--------------------------------GTGCGACTAACGACGCCCTTG  3.2 %
TCCGGTGCTGGTGCTACAGCCGG------------------------------TCGGTGCGACTAACGACGCCCTTG  1.8 %
TCCGGTGCTGGTGCTACAGCC------------------------------TCGGTGCGACTAACGACGCCCTTG  1.4 %
TCCGGTGCTGGTGCTACAGCCGG----------------------------------GTGCGACTAACGACGCCCTTG  1.2 %
TCCGGTGCTGGTGCTACAGCCGG------------------------------TCGGTGCGACTAACGACGCCCTTG  1.0 %
```

DNA Exp. 2 25.9%

*AtBON*1 gRNA1 *AtBON*1 gRNA2

```
TCCGGTGCTGGTGCTACAGCCGGCGTTGGTGGAAGTGGTTCCTCCGCCGCTCTCGGTGCGACTAACGACGCCCTTG  WT
TCCGGTGCTGGTGCTACAGCCGGCGTTGGTGGAAGTGGTTCCTCCGCCGCTCTCGGTGCGACTAACGACGCCCTTG  73.7 %
TCCGGTGCTGGTGCTACAGCCGG------------------------------TCGGTGCGACTAACGACGCCCTTG  23.6 %
TCCGGTGCTGGTGCTACAGCCGGCG--------------------------------GTGCGACTAACGACGCCCTTG  1.0 %
TCCGGTGCTGGTGCTACAGCCG------------------------------TCGGTGCGACTAACGACGCCCTTG  0.67 %
TCCGGTGCTGGTGCTACAGCCGG------------------------------TCTGGTGCGACTAACGACGCCCTTG  0.45 %
TCCGGTGCTGGTGCTACAGCCGGCGTTGGTGGAAGTGGTTCCTCCGCCGCTCTCGGTGCGACTAACGACGCCCTTG  0.42 %
```

DNA Exp. 3 26.5%

*AtBON*1 gRNA1 *AtBON*1 gRNA2

```
TCCGGTGCTGGTGCTACAGCCGGCGTTGGTGGAAGTGGTTCCTCCGCCGCTCTCGGTGCGACTAACGACGCCCTTG  WT
TCCGGTGCTGGTGCTACAGCCGGCGTTGGTGGAAGTGGTTCCTCCGCCGCTCTCGGTGCGACTAACGACGCCCTTG  73.5 %
TCCGGTGCTGGTGCTACAGCCGG------------------------------TCGGTGCGACTAACGACGCCCTTG  13.7 %
TCCGGTGCTGGTGCTACAGCCGGCGTTGGTGGAAGTGGTTCCTCCGCCGCTCTTCGGTGCGACTAACGACGCCCTTG  3.5 %
TCCGGTGCTGGTGCTACAGCCGGCGTTGGTGGAAGTGGTTCCTCCGCCGCTCATCGGTGCGACTAACGACGCCCTTG  1.1 %
```

FIGURE S1 | Comparison of *AtBON*1 editing efficiency via NHEJ by bNLSCas9 RNP or DNA transfection in Arabidopsis protoplasts. Indel (red) % of two additional biological experiments (Exp. 2 and 3) is shown by NGS analyses of the amplicons generated by PCR using genomic DNA isolated from transfected protoplasts. The *AtBON1* editing efficiency from three biological repeats is presented in FIGURE 2E.

A

AtPDS gRNA1 *AtPDS* gRNA2

RNP Exp. 1 63.6 %

```
GCGGAACAACGAGATGCTGACATGGCCAGAGAAAATAAAGTTTGCTATTGGACTTTTGCCAGCCATGGTCGGCG   WT
GCGGAACAACGAGATGCTGACATGGCCAGAGAAAATAAAGTTTGCTATTGGACTTTTGCCAGCCATGGTCGGCG   36 %
GCGGAACAACGAGATGCTG-----------------------------------------------GTCGGCG   25 %
GCGGAACAACGAGATGCTG--------------------------------------------TGGTCGGCG   21 %
GCGGAACAACGAGATGCTG-------------------------------------------GGTCGGCG   6.2 %
GCGGAACAACGAGATGCT-ACATGGCCAGAGAAAATAAAGTTTGCTATTGGACTTTTGCCAGCCATGGTCGGCG   2.2 %
GCGGAACAACGAGATGCT----------------------------------------------TGGTCGGCG   2.1 %
GCGGAACAACGAGATG------------------------------------------------GTCGGCG   1.8 %
GCGGAACAACGAGATGCT-ACATGGCCAGAGAAAATAAAGTTTGCTATTGGACTTTTGCCAGCCATTGGTCGGCG   1.4 %
GCGGAACAACGAGATGCTGTACATGGCCAGAGAAAATAAAGTTTGCTATTGGACTTTTGCCAGCCATGGTCGGCG   1.1 %
GCGGAACAACGAGATGCTGAACATGGCCAGAGAAAATAAAGTTTGCTATTGGACTTTTGCCAGCCATGGTCGGCG   1.0 %
GCGGAACAACGAGATG---ACATGGCCAGAGAAAATAAAGTTTGCTATTGGACTTTTGCCAGCCATGGTCGGCG   0.71 %
GCGGAACAACGAGATGCTGACATGGCCAGAGAAAATAAAGTTTGCTATTGGACTTTTGCCAGCCATTGGTCGGCG   0.66 %
```

AtPDS gRNA1 *AtPDS* gRNA2

DNA Exp. 1 7.6 %

```
GCGGAACAACGAGATGCTGACATGGCCAGAGAAAATAAAGTTTGCTATTGGACTTTTGCCAGCCATGGTCGGCG   WT
GCGGAACAACGAGATGCTGACATGGCCAGAGAAAATAAAGTTTGCTATTGGACTTTTGCCAGCCATGGTCGGCG   90.6 %
GCGGAACAACGAGATGCTG-----------------------------------------------GTCGGCG   2.1 %
GCGGAACAACGAGATGCTG-------------------------------------------GGTCGGCG   1.9 %
GCGGAACAACGAGATGCTG----------------------------------------------TGGTCGGCG   1.7 %
GCGGAACAACGAGATGCTGACATGGCCAGAGAAAATAAAGTTTGCTATTGGACTTTTACCAGCCATGGTCGGCG   1.8 %
GCGGAACAACGAGATGCTGACATGGCCAGAGAAAATAAAGTTTGCTATTGGACTTTTGCCAGCCATGGTCGGCG   0.89 %
GCGGAACAACGAGATGCTGACATGGCCAGAGAAAATAAAGTTTGCTATTGGACTTTTGCCAGCCATGGTCGGCG   0.80 %
GCGGAACAACGAGATGCTG-----------------------------------------------GGTCGGCG   0.80 %
```

B

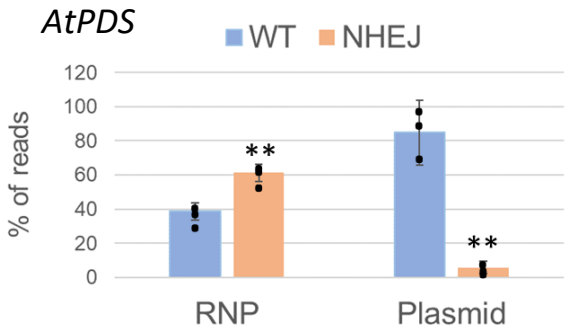

FIGURE S2 | Comparison of *AtPDS* editing efficiency via NHEJ by bNLSCas9 RNP or DNA transfection in Arabidopsis protoplasts. **(A)** Indel (red) % of one representative biological experiment is shown. **(B)** The *AtPDS* editing efficiency from three biological repeats (** $p < 0.01$; error bars, s.d., n=3).

A

AtFAD gRNA1 *AtFAD* gRNA2

RNP Exp. 1 63.8 %

```
CCGTGCGAGAAACCGCCTTTCTCGGTGGGAGATCTGAAGAAAGCAATCCCGCCGCATTGTTTCAAACGCTCAATCCC   WT
CCGTGCGAGAAACCGCCTTT----------------------------------------GTTTCAAACGCTCAATCCC   44.8 %
CCGTGCGAGAAACCGCCTTTCTCGGTGGGAGATCTGAAGAAAGCAATCCCGCCGCATTGTTTCAAACGCTCAATCCC   36.2 %
CCGTGCGAGAAACCGCCTT----------------------------------------GTTTCAAACGCTCAATCCC   10.6 %
CCGTGCGAGAAACCGCCTT-CTCGGTGGGAGATCTGAAGAAAGCAATCCCGCCGCATTGTTTCAAACGCTCAATCCC   1.7 %
CCGTGCGAGAAACCGCCTTTTCTCGGTGGGAGATCTGAAGAAAGCAATCCCGCCGCATTGTTTCAAACGCTCAATCCC   1.54 %
CCGTGCGAGAAACCGCCTTA----------------------------------------TGTTTCAAACGCTCAATCCC   1.40 %
CCGTGCGAGAAACCGCCTTATCTCGGTGGGAGATCTGAAGAAAGCAATCCCGCCGCATTGTTTCAAACGCTCAATCCC   1.37 %
CCGTGCGAGAAACCGCCT------------------------------------------GTTTCAAACGCTCAATCCC   1.23 %
CCGTGCGAGAAACCGCCTTTCTCGGTGGGAGATCTGAAGAAAGCAATCCCGCCGCATTGTTTCAAACGCTCAATCCC   0.99 %
```

AtFAD gRNA1 *AtFAD* gRNA2

DNA Exp. 1 10.6 %

```
CCGTGCGAGAAACCGCCTTTCTCGGTGGGAGATCTGAAGAAAGCAATCCCGCCGCATTGTTTCAAACGCTCAATCCC   WT
CCGTGCGAGAAACCGCCTTTCTCGGTGGGAGATCTGAAGAAAGCAATCCCGCCGCATTGTTTCAAACGCTCAATCCC   89.4 %
CCGTGCGAGAAACCGCCTTT----------------------------------------GTTTCAAACGCTCAATCCC   8.56 %
CCGTGCGAGAAACCGCCTT----------------------------------------GTTTCAAACGCTCAATCCC   1.37 %
CCGTGCGAGAAACCGCCTTA----------------------------------------TGTTTCAAACGCTCAATCCC   0.37 %
CCGTGCGAGAAACCGCCTTTTCTCGGTGGGAGATCTGAAGAAAGCAATCCCGCCGCATTGTTTCAAACGCTCAATCCC   0.33 %
```

B

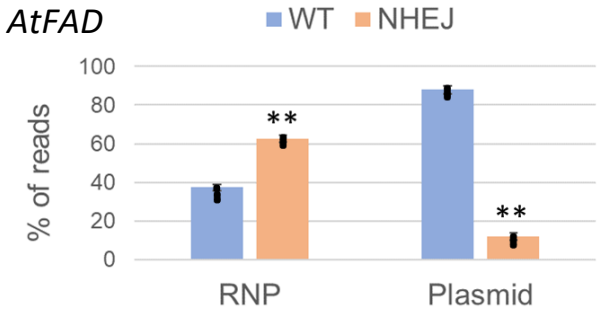

FIGURE S3 | Comparison of *AtFAD* editing effieincy via NHEJ by bNLSCas9 RNP or DNA transfection in Arabidopsis protoplasts. **(A)** Indel (red) % of one representative biological experiment is shown. **(B)** The *AtFAD* editing efficiency from three biological repeats (** $p < 0.01$; error bars, s.d., n=3).

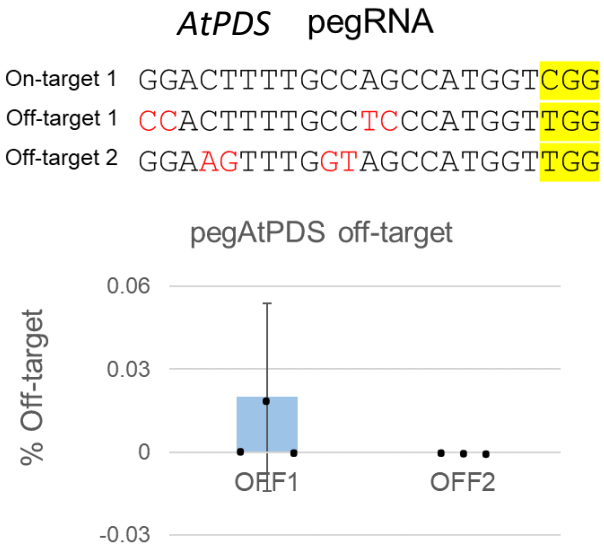

FIGURE S4 | Analyses of off-target site mutations mediated by PE RNP in Arabidopsis protoplasts. Red letter: predicted mismatch; Yellow highlight: PAM. Data from three biological repeats for two predicted off-target sites (*n*=3).

TABLE S1 | Primers used for T7 *in vitro* synthesis of gRNAs.

Gene	Forward primer sequence from 5' to 3'	Reverse primer from 5' to 3'
GFP	GAAATTAATACGACTCACTATAGGCGCTTCAAGGTGCACATGGGTTTTAGAGCTAGAAATAGCAAG	GCACCGACTCGGTGCCACTTTTTCAAGTTGATAACG
GFP	GAAATTAATACGACTCACTATAGGCTGAAGCACTGC*ACGCGT*AGTTTTAGAGCTAGAAATAGCAAG	GCACCGACTCGGTGCCACTTTTTCAAGTTGATAACG
AtBON 1	GAAATTAATACGACTCACTATAGGCTGGTGCTACAGCCGGCGTGTTTTAGAGCTAGAAATAGCAAG	GCACCGACTCGGTGCCACTTTTTCAAGTTGATAACG
AtBON 1	GAAATTAATACGACTCACTATAGGTCGTTAGTCGCACCGAGAGGTTTTAGAGCTAGAAATAGCAAG	GCACCGACTCGGTGCCACTTTTTCAAGTTGATAACG
AtALS	GAAATTAATACGACTCACTATAGGTTCCTCTTGTAGCAATCACGTTTTAGAGCTAGAAATAGCAAG	GCACCGACTCGGTGCCACTTTTTCAAGTTGATAACG
AtALS	GAAATTAATACGACTCACTATAGGTACCAATCATACGACGGTTTTAGAGCTAGAAATAGCAAG	GCACCGACTCGGTGCCACTTTTTCAAGTTGATAACG

TABLE S2 | Primers used for T7 *in vitro* synthesis of pegRNAs.

Gene	Forward primer sequence from 5' to 3'	Reverse primer from 5' to 3'
GFP	GAAATTAATACGACTCACTATAGGCTGAAGCACTGC*ACGCGT*AGTTTTAGAGCTAGAAATAGCAAG	AAGCACTGCACGCCGTAGGTGAAGCACCGACTCGGTGCCAC
GFP	GAAATTAATACGACTCACTATAGGCTGAAGCACTGC*ACGCGT*AGTTTTAGAGCTAGAAATAGCAAG	AAGCACTGCACGCCGTAGGTGAAGGTGCACCGACTCGGTGCCAC
GFP	GAAATTAATACGACTCACTATAGGCTGAAGCACTGC*ACGCGT*AGTTTTAGAGCTAGAAATAGCAAG	AAGCACTGCACGCCGTAGGTGAAGGTGGTGCACCGACTCGGTGCCAC
GFP	GAAATTAATACGACTCACTATAGGCTGAAGCACTGC*ACGCGT*AGTTTTAGAGCTAGAAATAGCAAG	AAGCACTGCACGCCGTAGGTGAAGGTGGTCAGCACCGACTCGGTGCCAC
GFP	GAAATTAATACGACTCACTATAGGCTGAAGCACTGC*ACGCGT*AGTTTTAGAGCTAGAAATAGCAAG	AAGCACTGCACGCCGTAGGTGAAGGTGGTCACGAGCACCGACTCGGTGCCAC
GFP	GAAATTAATACGACTCACTATAGGCTGAAGCACTGC*ACGCGT*AGTTTTAGAGCTAGAAATAGCAAG	AAGCACTGCACGCCGTAGGTGAAGGTGGTCACGAGGGTGCACCGACTCGGTGCCAC
GFP	GAAATTAATACGACTCACTATAGGCTGAAGCACTGC*ACGCGT*AGTTTTAGAGCTAGAAATAGCAAG	AAGCACTGCACGCCGTAGGTGAAGGTGGTCACGAGGGTGGGCCAGGGGCACCGACTCGGTGCCAC
AtPDS	GAAATTAATACGACTCACTATAGGGACTTTTGCCAGCCATGGTGTTTTAGAGCTAGAAATAGCAAG	TTTTGCCAGCCATGGTCGGCGGTTAGGCTTAGCACCGACTCGGTGC

TABLE S3 | Primers used for PCR amplification of the targeting sequences in the Arabidopsis genome.

Gene	Forward primer sequence from 5' to 3'	Reverse primer from 5' to 3'
AtBON 1	ATGCGTTGTTCGATCCAGGTACTTTTGAATC	ACGAGAGATAGATGATAGTGAAGGAAC
AtALS	ATGCCAGCAGAAGGATACGCTCGATCCTCAG	CTAAAAAGAAAGCTTCCTCAATAATCC
AtPDS	GTTGTTGCTGTTGGATTTACG	CACAGAAACTGTGAACTCAATAGCCTAC

TABLE S4 | Primers used for T7 *in vitro* synthesis of gRNAs.

Gene	Forward primer sequence from 5' to 3'	Reverse primer from 5' to 3'
AtPDS	GAAATTAATACGACTCACTATAGGGAACAACGAGATGCTGACAGTTTT AGAGCTAGAAATAGCAAG	GCACCGACTCGGTGCCACTTTTTCA AGTTGATAACG
AtPDS	GAAATTAATACGACTCACTATAGGGACTTTTGCCAGCCATGGTGTTTT AGAGCTAGAAATAGCAAG	GCACCGACTCGGTGCCACTTTTTCA AGTTGATAACG
AtFAD	GAAATTAATACGACTCACTATAGGTGCGAGAAACCGCCTTTCTGTTTT AGAGCTAGAAATAGCAAG	GCACCGACTCGGTGCCACTTTTTCA AGTTGATAACG
AtFAD	GAAATTAATACGACTCACTATAGGATTGAGCGTTTGAAACAATGGTTT TAGAGCTAGAAATAGCAAG	GCACCGACTCGGTGCCACTTTTTCA AGTTGATAACG

TABLE S5 | Primers used for PCR amplification of the targeting sequences in the *Arabidopsis* genome.

Gene	Forward primer sequence from 5' to 3'	Reverse primer from 5' to 3'
AtPDS	AAAAGTTGTTGCTGTTGGATTTACG	CACAGAAACTGTGAACTCAATAGCC
AtPDS	TTTTGTTGTTGCTGTTGGATTTACG	CACAGAAACTGTGAACTCAATAGCC
AtPDS	GGGGGTTGTTGCTGTTGGATTTACG	CACAGAAACTGTGAACTCAATAGCC
AtFAD	ATGCATGGGTGCAGGTGGAAGAATGC	AAGCATGAGGCTATAATGATGTC
AtFAD	ATCGATGGGTGCAGGTGGAAGAATGC	AAGCATGAGGCTATAATGATGTC
AtFAD	AGCTATGGGTGCAGGTGGAAGAATGC	AAGCATGAGGCTATAATGATGTC

TABLE S6 | Primers used for PCR amplification of predicted off-target site sequences.

Gene	Forward primer sequence from 5' to 3'	Reverse primer from 5' to 3'
AtBON1	GCTGTCGTTGCGCTTATC	CCTTCCTCAGTGTTGTTAGC
AtBON1	CCGTCTCACTCCCACCTC	GTCAAACCCTAGCGGCGGTG
AtBON1	GCTCCAGCTCCAGCTCCCTC	GTATTCGAACTTCACATAAG
AtPDS	CTTCCACACCCTTCTTGC	CAAGCCAGGCATCTTGCC
AtPDS	CCTAGGATTTCAAGTTGG	CAAAGATAACTTCTTGCTC
AtPDS	GATCTGTTTCTCTATAATC	GAATGTGTTCAAGCTGCG

DNA SEQUENCES

NHEJ GFP

GCGCTTCAAGGTGCACATGGAGGAAAAATCCATGGTGAGCAAGGGCGAGGAGCT
GTTCACCGGGGTGGTGCCCATCCTGGTCGAGCTGGACGGCGACGTAAACGGCCA

CAAGTTCAGCGTGTCCGGCGAGGGCGAGGGCGATGCCACCTACGGCAAGCTGAC
CCTGAAGTTCATCTGCACCACCGGCAAGCTGCCCGTGCCCTGGCCCACCCTCGTG
ACCACCTTCACCTACGGCGTGCAGTGCTTCAGCCGCTACCCCGACCACATGAAGC
AGCACGACTTCTTCAAGTCCGCCATGCCCGAAGGCTACGTCCAGGAGCGCACCAT
CTTCTTCAAGGACGACGGCAACTACAAGACCCGCGCCGAGGTGAAGTTCGAGGG
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CAACATCCTGGGGCACAAGCTGGAGTACAACTACAACAGCCACAACGTCTATATCA
TGGCCGACAAGCAGAAGAACGGCATCAAGGTGAACTTCAAGATCCGCCACAACAT
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GCCGCCGGGATCACTCACGGCATGGACGAGCTGTACAAGTAA
Black: GFP
Blue: target sequence
Red: PAM

HDR GFP
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GTACAAGTAA
Black GFP
Blue: target sequence
Red: PAM
Yellow highlight: 65TLR67

bNLSCas9

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A

Green: bipartite nuclear localization signal

Black underlined: *Cas9*
Green underlined: monopartite NLS
Black italic and underlined: 6XHIS tag

nCas9-RT

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Green: bipartite nuclear localization signal

Black underlined: *SpCas9*

Black bold: linker

Black italic M-MLV

Green underlined: monopartite NLS

Black italic and underlined: 6XHIS tag