1 **TITLE** 

Multigene Editing via CRISPR/Cas9 Guided by a Single-sgRNA Seed in
Arabidopsis

### 4 **RUNNING TITLE**

5 A Single sgRNA Seed for Multigene-editing

#### **6 KEYWORDS**

7 Single sgRNA Seed, CRISPR/Cas9, Multigene Editing, *AtRPL10* 

Summary We report that a single-sgRNA seed is capable of guiding CRISPR/Cas9 to 8 simultaneously edit multiple genes AtRPL10A, AtRPL10B and AtRPL10C in 9 10 Arabidopsis. Our results also demonstrate that it is possible to use CRISPR/Cas9 technology to create AtRPL10 triple mutants which otherwise cannot be generated by 11 conventional genetic crossing. Compared to other conventional multiplex 12 CRISPR/Cas systems, a single sgRNA seed has the advantage of reducing off-target 13 14 gene-editing. Such a single sgRNA seed-induced gene editing system might be also applicable to modify other homologous genes or even less-homologous sequences for 15 multiple gene-editing in plants and other organisms. 16

Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR)/CRISPR 17 -associated 9 (CRISRP/Cas9) is an adaptive immune mechanism that protects bacteria 18 and archaea from extrachromosomal DNA and viral invasions (Jinek et al. 2012). 19 CRISPR/Cas9 generates double-stranded breaks (DSBs) under specific guidance of a 20 21 single guide RNA (sgRNA). These DSBs can then be repaired either by homologous recombination or predominantly by non-homologous end-joining, which leads to 22 23 introduction of mutations such as nucleotide substitution, insertion or deletion into the targeted DNA molecules (Jinek et al. 2012; Cong et al. 2013). Such an ancient 24 defense has been exploited for efficient genome/gene editing in organisms across 25 kingdoms (Jinek et al. 2012; Cong et al. 2013; Fu et al. 2013; Mao et al. 2013; Gao 26 and Zhao, 2014; Ma et al. 2015; Yan et al., 2015; Kim et al., 2016; Shen et al., 2016). 27

Moreover, multiplex CRISPR/Cas9-based gene editing can also be simultaneously achieved through the use of different sgRNAs in animals and plants (Cong et al. 2013; Feng et al. 2014; Wang et al. 2015; Yan et al. 2015). It remains to be elucidated, however, whether multigene-editing via CRISPR/Cas9 can be directed by a single sgRNA seed.

To address this, we searched the Arabidopsis genome database and identified the 33 AtRPL10 family that includes three homologous members AtRPL10A (AT1G14320), 34 AtRPL10B (AT1G26910) and AtRPL10C (AT1G66580) coding for the Ribosomal 35 Protein Large 10 subunits. The three AtRPL10 genes reside at different loci on 36 Arabidopsis chromosome 1 (Figure S1), sharing 81-88% nucleotide (nt) identities, 37 38 and their protein products are 95-98% identical (Table S1) AtRPL10A and AtRPL10B are expressed in female and male reproductive organs whilst AtRPL10C is restricted 39 to pollen grains. The three multifunctional genes are involved in protein translation 40 and plant response to viral infection and abiotic stress (Falcone Ferreyra et al. 2013; 41 42 Zorzatto et al. 2015). Homozygous AtRPL10A T-DNA insertion mutation is lethal and RNAi of AtRPL10B affects plant growth, although AtRPL10C knockout results in no 43 phenotypic change (Falcone Ferreyra et al. 2010). Interestingly, genetic crosses can 44 generate AtRPL10A, AtRPL10B or AtRPL10C heterologous double, but not triple, 45 mutants in Arabidopsis (Falcone Ferreyra et al. 2013). 46

We generated an 'AtRPL10 sgRNA+CRISPR/Cas9' construct in pCAMBIA1300 47 (Figure 1A). The AtRPL10 sgRNA consists of an identical 19 nucleotides 48 (ATGTTGGTATGAAGAGGAA) targeting the three genes. However, the protospacer 49 50 adjacent motif (PAM) is AGG in AtRPL10B and AtRPL10C, but GGG in AtRPL10A (Figure 1B). A. thaliana ecotype Col-0 was transformed with the binary vector via the 51 floral dip method (Supplemental Materials and Methods). Four independent Line7, 52 Line9, Line10 and Line11 were created. Transgenic T1 plants from Lines7, 9 and 10 53 showed severe growth retardation and delayed flowering whilst Line11 had slightly 54 weaker growth compared to the wild-type Col-0 plants (Figure 1C, D). These lines 55

showed similar phenotypes to AtRPL10B RNAi plants, but differed from AtRPL10A 56 T-DNA insertion mutants or AtRPL10C knockout plants. To detect potential 57 multigene-editing events in these transgenic lines, we first analyzed the 58 sgRNA-targeted sequences using a high-fidelity PCR-RFLP (restriction fragment 59 length polymorphism) assay. An EarI site is located 4-9 nucleotides upstream of the 60 61 AtRPL10 sgRNA PAM sequence (Figure 1B), the region in which CRISPR/Cas9-mediated DSBs frequently occur (Jinek et al. 2012). We extracted 62 genomic DNA from transgenic and non-transformed Col-0 plant leaf tissues and 63 amplified the *AtRPL10* target sequences using gene-specific primers (Table S2). 64 Incomplete EarI-digestion of the resultant PCR products suggests that AtRPL10A and 65 AtRPL10C were successfully edited in Line9 (Figure 1E). 66

To further characterize multigene-editing in these transgenic lines, we cloned the PCR 67 products into pMD19-T (Supplemental Materials and Methods). Sequencing analyses 68 showed that nucleotide deletions and/or replacements were introduced into AtRPL10A, 69 70 AtRPL10B and AtRPL10C in all transgenic lines (Figures S2-13; Table S3; Dataset S1). However, the efficiency of multigene-editing of all target sequences was 71 relatively higher (Figure 1F; Figures S5-7) although varied among AtRPL10A (8.8%), 72 AtRPL10B (3.8%) and AtRPL10C (23.6%) in Line9 (Table 1). Using an alternative 73 assay, we identified 7 more (4 deletion and 3 substitution) mutations that were 74 introduced into AtRPL10B in Line9 (Figure 1G-I). In Line7 (Figures S2-4) and 75 Line10 (Figures S8-10), we detected nucleotide deletions in AtRPL10A or AtRPL10C 76 but not in AtRPL10B, whilst only point mutations were found in the three AtRPL10 77 78 genes in Line11 (Figure S11-13). In total we sequenced 1,222 clones and identified 75 different mutations, 37 of which were a deletion of 2 nucleotides. There were single 79 cases of 1nt or 4nt-deletions, and 36 cases of 1nt-substitution (Table S3). Nevertheless, 80 multiple deletion and/or point mutations introduced by a single-sgRNA seed-directed 81 CRISPR/Cas9 were correlated with the abnormal phenotypes in the transgenic lines 82 (Figure 1C). 83

Multiplex gene editing through CRISPR/Cas9 that is directed by a number of different 84 sgRNAs has been previously reported in animals and plants (Cong et al. 2013; Feng et 85 al. 2014; Wang et al. 2015; Yan et al. 2015). In this letter, we show that a 86 single-sgRNA seed is capable of guiding CRISPR/Cas9 to edit multiple genes in 87 Arabidopsis. Secondly, we demonstrate that it is possible to use CRISPR/Cas9 88 technology to create AtRPL10A/B/C triple mutants which otherwise cannot be 89 generated by conventional genetic crossing. Thirdly, we observe that most of 90 91 mutations resulted from the single-sgRNA seed-guided CRISPR-Cas9 are 2nt-deletion 92 or 1nt-substitution within the sgRNA-target sequences. This differs from a previous report that mutations induced by CRISPR/Cas9 were predominantly 1nt-insertion and 93 short deletions of nucleotides (Feng et al. 2014), but consistent with others (Wang et 94 al. 2015; Yan et al., 2015). Fourthly, the different AtRPL10A/B/C-editing efficiencies 95 (Table 1), particularly in Line9, suggest that chromosomal locations of genes along 96 with the contexts of their surrounding-sequences, heterochromatin architectures 97 and/or DNA/histone methylation may affect the CRISPR/Cas9 system for editing 98 99 multiple homologous genes (Kleinstiver et al. 2015). Nevertheless, Line9 may prove to be a valuable model to investigate positional effects on the ability of single 100 sgRNA-directed CRISPR/Cas9 to target and edit multiple genes in plants. Lines7, 10 101 and 11 may be also useful to explore why the single-sgRNA directed CRISPR/Cas9 102 system preferably causes nucleotide substitution, rather than deletion mutations in 103 target genes. It is interesting to note that all deletion mutations created in our 104 105 transgenic lines result from removal of 1, 2 or 4 nucleotides, causing frameshifts of the target genes. Compared to conventional multiplex CRISPR/Cas systems (Fu et al. 106 107 2013), a single-sgRNA seed has the advantage of reducing off-target gene-editing. 108 This approach is also applicable for the modification of other homologous genes. Moreover, considering how CRISPR/Cas9 recognizes canonical or non-canonical 109 PAMs such as NGG, NGA, NGCG, TTN and YTN (Kleinstiver et al. 2015; Zetsche et 110 al. 2015; Fonfara et al. 2016) as well as how sgRNAs interact with their target 111 sequences (Jinek et al. 2012), it should also be possible to design a single 112 'less-stringent' sgRNA seed that may target less-homologous sequences for 113

114 multigene-editing in plants and other organisms.

# 115 **ACKNOWLEDGMENTS**

116	We thank Jian-kang Zhu for his kind gifts of AtU6-26-sgRNA-SK and 35S-Cas9-SK
117	plasmids. We are grateful to Chun Wang for her suggestions and technical support.
118	This work was supported by grants from the National Science Foundation of China
119	(NSFC, 31200913), the Ministry of Agriculture of the People's Republic of China
120	National Transgenic Program (2016ZX08009001-004), the China Scholarship Council
121	(201709645003), NSFC (31370180; 31601765) and the Zhejiang Provincial Natural
122	Science Foundation (LY15C140006, LY14C010005).
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Z.Y. and Y.H. designed experiments; Z.Y. and Q.C. performed all experiments; W.C.
and X.Z. analyzed bioinformatics data; J.N., F.M., P.Z., M.Z., X.W. and N.S.
performed research. S.J. analysed the data and helped write the paper; Z.Y. and Y.H.
wrote the paper.

- **REFERENCES** 140 Cong L, Ran FA, Cox D, Lin S, Barretto R, Habib N, Hsu PD, Wu X, Jiang W, Marraffini LA, 141 142 Zhang F (2013) Multiplex genome engineering using CRISPR/Cas systems. Science 143 339:819-823. Falcone Ferreyra ML, Casadevall R, Luciani MD, Pezza A, Casati P (2013) New evidence for 144 differential roles of 110 ribosomal proteins from Arabidopsis. Plant Physiol 163:378-391. 145 146 Falcone Ferreyra ML, Pezza A, Biarc J, Burlingame AL, Casati P (2010) Plant L10 ribosomal proteins have different roles during development and translation under ultraviolet-B stress. 147 Plant Physiol 153:1878-1894. 148 Feng Z, Mao Y, Xu N, Zhang B, Wei P, Yang DL, Wang Z, Zhang Z, Zheng R, Yang L, Zeng 149 L, Liu X, Zhu JK (2014) Multigeneration analysis reveals the inheritance, specificity, and 150 151 patterns of CRISPR/Cas-induced gene modifications in Arabidopsis. Proc Natl Acad Sci **USA** 111:4632-4637. 152 Fonfara I, Richter H, Bratovič M, Le Rhun A, Charpentier, E (2016) The CRISPR-associated 153 154 DNA-cleaving enzyme Cpf1 also processes precursor CRISPR RNA. Nature 532:517-521. 155 Fu Y, Foden JA, Khayter C, Maeder ML, Reyon D, Joung JK, Sander JD (2013) 156 High-frequency off-target mutagenesis induced by CRISPR-Cas nucleases in human cells. 157 158 Nat Biotechnol 31:822-826. Gao Y. Zhao Y (2014) Self-processing of ribozyme-flanked RNAs into guide RNAs in vitro and in 159 vivo for CRISPR-mediated genome editing. J Integr Plant Biol. 56:343-349. 160 161 Jinek M, Chylinski K, Fonfara I, Hauer M, Doudna JA, Charpentier E (2012) A programmable dual-RNA-guided DNA endonuclease in adaptive bacterial immunity. 162 Science 337:816-821. 163
- Kim H, Kim ST, Ryu J, Choi MK, Kweon J, Kang BC, Ahn HM, Bae S, Kim J, Kim JS, Kim
   SG (2016) A simple, flexible and high-throughput cloning system for plant genome editing
   via CRISPR-Cas system. J Integr Plant Biol. 58:705-712.
  - 6

- 167 Kleinstiver BP, Prew MS, Tsai SQ, Topkar VV, Nguyen NT, Zheng Z, Gonzales AP, Li Z,
- Peterson RT, Yeh JR, Aryee MJ, Joung JK (2015) Engineered CRISPR-Cas9 nucleases
  with altered PAM specificities. Nature 523:481-485.
- 170 Ma X, Zhang Q, Zhu Q, Liu W, Chen Y, Qiu R, Wang B, Yang Z, Li H, Lin Y, Xie Y, Shen R,
- 171 Chen S, Wang Z, Chen Y, Guo J, Chen L, Zhao X, Dong Z, Liu YG (2015) A robust
- 172 CRISPR/Cas9 system for convenient, high-efficiency multiplex genome editing in
- 173 monocot and dicot plants. **Mol Plant** 8:1274-1284.
- Mao Y, Zhang H, Xu N, Zhang B, Gou F, Zhu JK (2013) Application of the CRISPR-Cas
  system for efficient genome engineering in plants. Mol Plant 6:2008-2011.
- Shen L, Wang C, Fu Y, Wang J, Liu Q, Zhang X, Yan C, Qian Q, Wang K (2016) QTL editing
   confers opposing yield performance in different rice varieties. J Integr Plant Biol. doi:
   10.1111/jipb.12501.
- Wang ZP, Xing HL, Dong L, Zhang HY, Han CY, Wang XC, Chen QJ (2015) Egg
   cell-specific promoter-controlled CRISPR/Cas9 efficiently generates homozygous mutants
- for multiple target genes in Arabidopsis in a single generation. **Genome Biol.** 16:144.
- 182 Yan L, Wei S, Wu Y, Hu R, Li H, Yang W, Xie Q (2015) High-Efficiency Genome Editing in
- 183 Arabidopsis Using YAO Promoter-Driven CRISPR/Cas9 System. Mol Plant. 8:1820-1823.
- Yan M, Zhou SR, Xue HW (2015) CRISPR Primer Designer: Design primers for knockout
   and chromosome imaging CRISPR-Cas system. J Integr Plant Biol. 57:613-617.
- 186 Zetsche B, Gootenberg JS, Abudayyeh OO, Slaymaker IM, Makarova KS, Essletzbichler P,
- 187 Volz SE, Joung J, van der Oost J, Regev A, Koonin EV, Zhang F (2015) Cpf1 is a single
- 188 RNA-guided endonuclease of a class 2 CRISPR-Cas system. Cell 163:759-771.
- 189 Zorzatto C, Machado JP, Lopes KV, Nascimento KJ, Pereira WA, Brustolini OJ, Reis PA,
- 190 Calil IP, Deguchi M, Sachetto-Martins G, Gouveia BC, Loriato VA, Silva MA, Silva FF,
- 191 Santos AA, Chory J, Fontes EP (2015) NIK1-mediated translation suppression functions as
- a plant antiviral immunity mechanism. **Nature** 520:679-682.

# 193 SUPPORTING INFORMATION

194 Additional Supporting Information is available.

195 196 197 198 199 200	Figure S1. Physical Positions of AtRPL10A, AtRPL10B and AtRPL10C in Arabidopsis         Chromosome 1.         AtRPL10A (AT1G14320):       4,888,214 – 4,889,661;         AtRPL10B (AT1G26910):       9,321,650 – 9,322,965;         Chromosome Centromere:       14,899,838 – 14,906,596;         AtRPL10C (AT1G66580):       24,839,165 – 24,840,612.
201 202 203 204	<ul> <li>Figure S2. Triple Gene Editing in Line7.</li> <li>(A) <i>AtRPL10A</i> sequences of individual clones. PAM sequence is boxed and the edited nucleotides are highlighted red.</li> <li>(B) Representative chromatograms of <i>AtRPL10A</i> sequences with edited nucleotides</li> </ul>
205 206 207 208	<ul> <li>Figure S3. Triple Gene Editing in Line7.</li> <li>(A) <i>AtRPL10B</i> sequences of individual clones. PAM sequence is boxed and the edited nucleotides are highlighted red.</li> <li>(B) Representative chromatograms of <i>AtRPL10B</i> sequences with edited nucleotides.</li> </ul>
209 210 211 212	<ul> <li>Figure S4. Triple Gene Editing in Line7.</li> <li>(A) <i>AtRPL10C</i> sequences of individual clones. PAM sequence is boxed and the edited nucleotides are highlighted red.</li> <li>(B) Representative chromatograms of <i>AtRPL10C</i> sequences with edited nucleotides.</li> </ul>
213 214 215 216	<ul> <li>Figure S5. Triple Gene Editing in Line9.</li> <li>(A) <i>AtRPL10A</i> sequences of individual clones. PAM sequence is boxed and the edited nucleotides are highlighted red.</li> <li>(B) Representative chromatograms of <i>AtRPL10A</i> sequences with edited nucleotides.</li> </ul>
217 218 219 220	<ul> <li>Figure S6. Triple Gene Editing in Line9.</li> <li>(A) <i>AtRPL10B</i> sequences of individual clones. PAM sequence is boxed and the edited nucleotides are highlighted red.</li> <li>(B) Representative chromatograms of <i>AtRPL10B</i> sequences with edited nucleotides.</li> </ul>
221 222 223 224	<ul> <li>Figure S7. Triple Gene Editing in Line9.</li> <li>(A) <i>AtRPL10C</i> sequences of individual clones. PAM sequence is boxed and the edited nucleotides are highlighted red.</li> <li>(B) Representative chromatograms of <i>AtRPL10C</i> sequences with edited nucleotides.</li> </ul>
225 226 227 228	<ul> <li>Figure S8. Triple Gene Editing in Line10.</li> <li>(A) <i>AtRPL10A</i> sequences of individual clones. PAM sequence is boxed and the edited nucleotides are highlighted red.</li> <li>(B) Representative chromatograms of <i>AtRPL10A</i> sequences with edited nucleotides.</li> </ul>
229 230	<b>Figure S9. Triple Gene Editing in Line10.</b> (A) <i>AtRPL10B</i> sequences of individual clones. PAM sequence is boxed and the edited nucleotides

- are highlighted red.
- (B) Representative chromatograms of *AtRPL10B* sequences with edited nucleotides.
- 233 Figure S10. Triple Gene Editing in Line10.
- (A) AtRPL10C sequences of individual clones. PAM sequence is boxed and the edited nucleotides
- are highlighted red.
- (B) Representative chromatograms of *AtRPL10C* sequences with edited nucleotides.
- 237 Figure S11. Triple Gene Editing in Line11.
- 238 (A) AtRPL10A sequences of individual clones. PAM sequence is boxed and the edited nucleotides
- are highlighted red.
- 240 (B) Representative chromatograms of *AtRPL10A* sequences with edited nucleotides.
- 241 Figure S12. Triple Gene Editing in Line11.
- 242 (A) *AtRPL10B* sequences of individual clones. PAM sequence is boxed and the edited nucleotides243 are highlighted red.
- 244 (B) Representative chromatograms of *AtRPL10B* sequences with edited nucleotides.
- 245 Figure S13. Triple Gene Editing in Line11.
- 246 (A) *AtRPL10C* sequences of individual clones. PAM sequence is boxed and the edited nucleotides247 are highlighted red.
- 248 (B) Representative chromatograms of *AtRPL10C* sequences with edited nucleotides.
- 249 Table S1. Comparisons of AtRPL10 Genes and Their Protein Products
- 250 Table S2. Primers Used in This Study
- Table S3. Multigene Editing and Their Impacts on Protein Sequences in CRISPR/Cas9
   Transgenic Lines
- 253 Dataset S1. Sequences of the PCR Products for the Three *AtRPL10* Genes.
- 254 (A-B) Restriction fragment length polymorphism (RFLP) analysis of AtRPL10A (A), AtRPL10B
- 255 (B) AtRPL10C (C). Sequences corresponding to the 'seed' sgRNA are indicated in lowercase. The
- 256 *EarI* digestion site () is indicated and its recognition sequence is underlined.

### 257 **FIGURE LEGEND**

#### 258 Figure 1. A Single sgRNA Seed Directs CRISPR/Cas9 to Simultaneously Edit

#### 259 Three AtRPL10 Homologous Genes.

- 260 (A) Schematic of the single sgRNA seed and CRISPR/Cas9 consturct in the binary vector
- 261 pCAMBIA1300. Nucleotides corresponding to the sgRNA seed sequence are underlined. The
- 262 AtU6-26 promoter (arrow), sgRNA and the scaffold, enhanced 35S promoter (arrow), NLS
- 263 (nuclear localization signal)-tagged Cas9, hygromycin (HYG) as well as the right and left
- borders (RB and LB) in the binary vector are indicated.
- 265 (B) Comparison of the sgRNA seed-targeted AtRPL10 gene sequences. The EarI site is

- underlined. The PAM sequences are highlighted red. Nucleotide coordinates are indicated.
- 267 (C) Phenotypes of transgenic plants of four independent lines. Bar = 3cm in Line7, Line9,

Line 10 and Line 11. Bar = 5 cm in Col-0.

- 269 (D) Confirmation of plant transformation. The Cas9 gene was detected in four transgenic lines
- as indicated. A BM2000 DNA ladder (Marker) as well as the size and position of the Cas9
- transgene PCR fragment are indicated.
- 272 (E) PCR-RFLP assay of multiple gene-editing in four transgenic lines. Gene-specific PCR
- products were digested with *Ear*I. Incomplete digestion shows three clear bands, indicating
  that successful editing of *AtRPL10A* and *AtRPL10C* in Line9. A BM2000 DNA ladder
- 275 (Marker) was included in gel electrophoresis.
- (F) Sequencing analysis of multiple gene-editing in Line9. Representative sequencings show
  indels in *AtRPL10A*, *AtRPL10B* and *AtRPL10C*. The sgRNA target sequences are shown in
  lowercase.
- (G-I) PCR-RFLP and sequencing assays of *AtRPL10B* editing in Line9. After *Ear*I digestion,
  residual DNA in the postion of the red-box was extracted from the argarose gel (G) and
  subcloned for sequencing analysis (H). A BM2000 DNA ladder (M) was included in gel
  electrophoresis. Sequences of 19 individual clones for *AtRPL10B* were aligned, and mutations
  with two nucleotide-deletion (red arrow) or single nucleotide-substitution (highlighted red)
  are indicated (I). RD stands for restriction endonuclease digestion.

## 285 **TABLE**

# **Table 1. Summary of Multigene Editing Efficiency**\*.

Treneganie	AtRPL10A		AtRPL10B		AtRPL10C	
Transgenic Lines	Deletion	Point	Deletion	Point	Deletion	Point
Lines	Deletion	Mutation	Deletion	Mutation		Mutation
Line 7	1/95	2/95	0/99	5/99	0/112	6/112
Line 9	7/102	2/102	1/105	3/105	25/123	4/123
Line 10	0/106	3/106	0/101	2/101	1/96	1/96
Line 11	0/93	2/93	0/109	4/109	0/62	2/62

<sup>\*</sup>The number of CRISPR/Cas9 edited sequences (clones) out of the total number of sequenced

samples for the three *AtRPL10* genes in each of the transgenic lines.