

# 1 **An inducible genome editing system for plants**

2 **Xin Wang<sup>1,2</sup>, Lingling Ye<sup>1,2</sup>, Robertas Ursache<sup>3</sup>, Ari Pekka Mähönen<sup>1,2,\*</sup>**

3 1. Institute of Biotechnology, HiLIFE, University of Helsinki, Helsinki 00014, Finland

4 2. Organismal and Evolutionary Biology Research Programme, Faculty of Biological and  
5 Environmental Sciences, and Viikki Plant Science Centre, University of Helsinki, Helsinki 00014,  
6 Finland

7 3. Department of Plant Molecular Biology, Biophore, Campus UNIL-Sorge, University of Lausanne  
8 CH-1015, Lausanne, Switzerland

9 \*Author for correspondence: [AriPekka.Mahonen@helsinki.fi](mailto:AriPekka.Mahonen@helsinki.fi)

10 ORCID IDs: 0000-0002-8982-9848 (XW), 0000-0003-3834-862X (LLY), 0000-0002-3803-253X (RU),  
11 0000-0001-6051-866X (APM)

12

## 13 **ABSTRACT**

14 Conditional manipulation of gene expression is a key approach to investigating the primary function  
15 of a gene in a biological process. While conditional and cell-type specific overexpression systems  
16 exist for plants, there are currently no systems available to disable a gene completely and  
17 conditionally. Here, we present a novel tool with which target genes can be efficiently conditionally  
18 knocked out at any developmental stage. The target gene is manipulated using the CRISPR-Cas9  
19 genome editing technology, and conditionality is achieved with the well-established estrogen-  
20 inducible XVE system. Target genes can also be knocked-out in a cell-type specific manner. Our  
21 tool is easy to construct and will be particularly useful for studying genes which have null-alleles  
22 that are non-viable or show strong developmental defects.

23

24

## 25 MAIN TEXT

26 Studies of gene function typically rely on phenotypic analysis of loss-of-function mutants.  
27 However, mutations may lead to gametophytic or embryonic lethality, or early developmental  
28 defects, impeding studies in postembryonic plants. The genome of the model species *Arabidopsis*  
29 contains a substantial number of such essential genes, though the precise number remains  
30 unknown<sup>1</sup>. Developing a tool that enables conditional and cell-type specific gene disruption is  
31 therefore of great value for comprehensively investigating gene function in specific developmental  
32 or physiological processes.

33 Different strategies have been pursued for this purpose. One widely applied approach is the  
34 inducible expression of silencing small RNAs<sup>2,3</sup>. However, this results in only a partial reduction of  
35 transcript levels, which may hinder a full investigation of gene function. Furthermore, since small  
36 RNAs can be mobile<sup>4</sup>, constraining the knockdown effect to a given cell-type is challenging. These  
37 limitations can be overcome by using the Cre/lox based clonal deletion system, which provides the  
38 possibility of a full knockout together with cell-type specificity. However, this method relies on  
39 complicated genetic engineering and has thus remained a rather marginal technique<sup>5,6,7</sup>.

40 The CRISPR-Cas9 system consists of components derived from the prokaryote adaptive immune  
41 system which have been modified for use as a genome editing toolkit in eukaryotes. The  
42 endonuclease activity of Cas9 produces double-strand breaks (DSB) in DNA when directed to a  
43 target by a single guide RNA (sgRNA). The subsequent error-prone DSB repair mediated by non-  
44 homologous end joining facilitates knockout generation. Thus far, CRISPR-Cas9 has been used in  
45 plants to generate stable knockouts<sup>8</sup> and somatic knockouts at fixed developmental stages by  
46 driving Cas9 expression with tissue-specific promoters<sup>9</sup>. By integrating the well-established  
47 CRISPR-Cas9 technology<sup>10</sup> with an XVE-based cell-type specific inducible system<sup>11,12</sup>, we

48 developed an Inducible Genome Editing (IGE) system in *Arabidopsis* which enables efficient  
49 generation of target gene knockouts in desired cell types and at desired times.

50 To achieve this, we first generated a fusion of a small nucleolar RNA promoter and an sgRNA  
51 (*pAtU3/6-sgRNA*) in two sequential PCR amplification steps (Fig. 1a). The fusion was then cloned  
52 into the *p2PR3-Bsa I-ccdB-Bsa I* entry vector (3<sup>rd</sup> box) by Golden Gate cloning<sup>10</sup>. This method  
53 allows simultaneous cloning of several *pAtU3/6-sgRNA* fragments, if needed. Next, we recombined  
54 a plant-codon optimized *Cas9p*<sup>10</sup> into *pDONR 221z* (2<sup>nd</sup> box). Finally, the IGE binary vector was  
55 generated in a single MultiSite Gateway LR reaction by combining an estrogen-inducible promoter  
56 (1<sup>st</sup> box)<sup>11</sup>, *Cas9p* (2<sup>nd</sup> box), *pAtU3/6-sgRNA* (3<sup>rd</sup> box) and a plant-compatible destination vector<sup>11,13</sup>  
57 (Fig. 1a). To facilitate screening of transformed seeds, we also generated two non-destructive  
58 fluorescent screening vectors (Supplementary Fig. 1). The availability of a large collection of cell-  
59 type specific or ubiquitous inducible promoters<sup>11</sup> and of destination vectors with different selection  
60 markers<sup>11,13</sup> makes the IGE system quite versatile. In summary, an IGE construct can be generated  
61 in two cloning steps: first, generating a *pAtU3/6-sgRNA* entry vector by Golden Gate cloning and  
62 then performing an LR reaction.

63 Next, we tested the IGE system in the *Arabidopsis* root meristem (RM) by targeting well-  
64 established regulatory genes that are essential for RM development. In the RM, a subset of  
65 AP2/EREBP family transcription factors, including *PLETHORA1* (*PLT1*) and *PLT2*, form gradients  
66 with maxima at the quiescent center (QC) to drive the transition from stem cells to differentiated  
67 cells<sup>14-16</sup>. The double mutant *plt1,2* exhibits a fully differentiated RM 6-8 days after germination<sup>14</sup>,  
68 which can be rescued by complementing it with *gPLT2-3xYFP*<sup>16</sup>. The fused 3xYFP restricts the  
69 mobility of *PLT2*<sup>16</sup>, making it possible to observe cell-specific effects of editing *PLT2*. We designed  
70 four sgRNAs to target *PLT2* in the *gPLT2-3xYFP; plt1,2* background (Supplementary Fig. 2a,b).  
71 *Cas9p* or nuclease-dead *Cas9p* (*dCas9p*) were transcribed under the inducible, ubiquitous promoter  
72 *35S-XVE* (*ip35S*)<sup>11</sup>. While induction of *dCas9p* had no effect on *PLT2-3xYFP* levels (Fig. 1d),

73 *Cas9p* induction led to a weakening of the YFP signal almost in every transformant (Fig. 1e). YFP  
74 fluorescence was initially reduced in the root cap and occasionally in the epidermis or stele.  
75 Prolonged induction gradually abolished the YFP signal and led to RM differentiation after 8-10  
76 days of induction (Fig. 1e and Supplementary Table 1), similar to the uncomplemented *plt1,2*  
77 mutant<sup>14</sup>.

78 The requirement of Cas9p nuclease activity for the disappearance of YFP fluorescence suggests that  
79 genome editing of *PLT2* caused a homozygous frame-shift mutation in *gPLT2-3xYFP*. PCR was  
80 performed to test whether a DNA fragment was deleted within the four target sites in *PLT2*.  
81 Intriguingly, only a single truncated band was detected aside from the expected WT band,  
82 corresponding to fragment deletion between the first and last targets. Sanger sequencing confirmed  
83 this deletion (Supplementary Fig. 2b-d). Further experiments revealed that constructs with just a  
84 single sgRNA could achieve equal efficiency in editing *gPLT2-3xYFP*; however, the efficiency  
85 strongly depended on the SnoRNA promoter used, with *AtU3b* and *AtU6-29* being the most  
86 efficient promoters, at least in the *Arabidopsis* RM (Supplementary Fig. 3 and Supplementary Table  
87 1). Since *AtU3b* and *AtU6-29* were used to drive the expression of sgRNA1 and sgRNA4,  
88 respectively, corresponding to the first and last targets in *PLT2*, this seems to explain the prevalence  
89 a deletion between these two positions (Supplementary Fig. 2). In summary, the IGE system enables  
90 efficient conditional genome editing even with a single sgRNA; however, if a complementary  
91 reporter line for the target gene does not exist, it is advisable to use two or more sgRNAs to  
92 generate a deletion which can be easily detected by PCR.

93 Next, we investigated whether the IGE system can be used to induce genome editing in a cell-type  
94 specific manner. We tested four inducible promoters: *pWOL-XVE* (*ipWOL*), *pWOX5-XVE*  
95 (*ipWOX5*), *pSCR-XVE* (*ipSCR*), and *pWER-XVE* (*ipWER*)<sup>11</sup>, the expression of which, together,  
96 covers most of the cell types in the RM. *Cas9p-tagRFP* was used to monitor promoter activity.  
97 Constructs were transformed into *gPLT2-3xYFP; plt1,2*. Along with promoter-specific Cas9-tagRFP

98 expression, we observed a corresponding dampening of the YFP signal in the respective domains  
99 after one day of induction (Fig. 2a). Consistent with the role of *PLT2* in promoting stem cell  
100 maintenance and QC specification, inducible editing in promoter-specific tissues caused premature  
101 cell expansion or differentiation of the endodermis, QC, or epidermis/lateral root cap (LRC) after 3  
102 days of induction (Fig. 2b). This reflects the cell-autonomous function of *PLT2* in maintaining an  
103 undifferentiated cell state. In addition to QC differentiation, we observed a shift in *ipWOX5*  
104 promoter activity towards the provascular, which resulted in a larger area lacking the YFP signal  
105 (Fig. 2b; left panel in *ipWOX5*). The QC and adjacent provascular cells gained columella cell  
106 identity, as revealed by the accumulation of starch granules (Fig. 2b; right panels in *ipWOX5*).  
107 These results indicate that new QC cells were re-specified from provascular cells following  
108 differentiation of the original QC, and the consequent re-specification and differentiation of the QC  
109 gradually led to a larger domain without YFP. These results are consistent with experiments in  
110 which laser ablation of the QC leads to re-specification of a new QC from provascular cells<sup>17</sup>.

111 We found that genome editing correlates strongly with Cas9p expression (Supplementary Fig. 4, 5).  
112 The expression level, the timing of induction and the expression region of Cas9-tagRFP determined  
113 editing performance in independent transformants. In addition, analysis showed that the editing  
114 capability of the IGE system is stably transmitted to the T2 generation (Supplementary Fig. 6).

115 To test whether the IGE system can edit other loci, we targeted a key gene encoding a cell cycle  
116 regulator, *RETINOBLASTOMA-RELATED (RBR)*<sup>7,18</sup>. The *RBR* null allele is gametophyte-lethal<sup>18</sup>.  
117 Previous conditional knockdowns and clonal deletion experiments have shown that RBR has a role  
118 in restricting stem cell division in the RM<sup>6,7,19</sup>. RBR-IGE constructs were transformed into a  
119 background in which *RBR-YFP* complements an *RBR* artificial microRNA line, *35S:amiGORBR*  
120 (*amiGORBR*)<sup>19</sup>. After one day of induction, we observed loss of YFP specifically in the respective  
121 promoter domains (Fig. 2c). Three days of induction led to cell overproliferation in the QC, LRC  
122 and endodermis, recapitulating the reported phenotype<sup>6,7,19</sup> (Fig. 2d).

123 When inducing Cas9p-tagRFP, we found that *ip35S* was not expressed ubiquitously but instead  
124 preferentially in the root cap and sometimes in the epidermis or stele (Fig. 2a and Supplementary  
125 Fig. 4). This pattern matches the domain of reduced RBR-YFP (Supplementary Fig. 7b) and PLT2-  
126 3xYFP expression (Fig. 1e and Supplementary Fig. 4) after a 1-day induction. After long-term  
127 induction of *ip35S* or *ipWER*, PLT2-3xYFP expression decreased outside the promoter-active  
128 region, in contrast to the effect on RBR (Fig. 1e, Fig. 2b, 2d and Supplementary Fig. 7c). These  
129 results suggest that loss of *PLT2* in the epidermis and LRC leads to endogenous, non-cell-  
130 autonomous, negative feedback regulation of *PLT2* expression in the rest of the RM, leading to  
131 differentiation. In addition, our results confirm the reported cell-autonomous function of RBR<sup>6</sup>.

132 To further demonstrate the wide applicability of the IGE system, we selected *GNOM* as a target.  
133 *GNOM* encodes a brefeldin A (BFA) sensitive ARF guanine-nucleotide exchange factor (ARF-GEF)  
134 that plays essential roles in endosomal structural integrity and trafficking<sup>20</sup>. *GNOM* has been  
135 implicated in polar localization of auxin efflux carrier (PINs), but previous studies relied on high-  
136 concentration BFA treatments or on hypomorphic alleles<sup>21,22</sup> because the null allele displays severe  
137 overall defects<sup>23,24</sup>. To test the response of PIN1 to the loss of *GNOM*, we made a construct using  
138 the *ipWOL* promoter to target *GNOM* in the vasculature and transformed it into both *GN-GFP*<sup>20</sup> and  
139 *PINI-GFP*<sup>25</sup> backgrounds. Following GN-GFP signal disappearance, most transformants displayed  
140 short roots, agravitropic growth and reduced lateral root formation 10 days after germination on  
141 induction plates (Supplementary Fig. 8, 9), a similar phenotype to the *gnom* mutant<sup>23</sup>. We then  
142 focused on PIN1 localization. Following 3 days of induction, PIN1 lost basal polarity and its  
143 expression was strongly inhibited (Supplementary Fig. 9), confirming the role of *GNOM* in driving  
144 basal localization of PIN1<sup>21,22</sup>.

145 When inducing editing of *PLT2*, *RBR* or *GNOM* with *ip35S* or *ipWOL*, we observed cell death in  
146 the proximal stem cells of the RM, which have been shown to be sensitive to genotoxic stress<sup>26</sup>  
147 (Supplementary Fig. 10a,b). Although it has been reported that *RBR* silencing causes DNA damage

148 and cell death<sup>27</sup>, *PLT2* and *GNOM* have not been shown to regulate cell death before. It is thus  
149 likely that Cas9p-induced DSBs activate downstream DNA damage signals which trigger a cell  
150 death response in proximal stem cells.

151 Next, we tested whether a single YFP-targeting IGE construct can be used to edit several different  
152 YFP-containing complementing lines. When targeting fused *YFP* in *gPLT2-3xYFP; plt1,2* and *RBR-*  
153 *YFP; amiGORBR* backgrounds, we found a strong reduction in YFP followed by characteristic  
154 developmental defects (Supplementary Fig. 11), similar to targeting *PLT2* and *RBR* directly (Fig.  
155 2b, 2d). For example, in *gPLT2-3xYFP; plt1,2*, editing *YFP* in the QC caused QC differentiation,  
156 though at a lower frequency than when *PLT2* was targeted. Likewise, we observed LRC  
157 overproliferation when targeting *YFP* in *RBR-YFP; amiGORBR*. However, unlike when *RBR* was  
158 targeted, the YFP signal also decreased in the rest of the RM by an unknown mechanism  
159 (Supplementary Fig. 11c). Many fluorescent-tagged lines complementing important genes are  
160 available, so targeting reporter-encoding genes might represent a broadly applicable approach for  
161 gene function studies. Furthermore, targeting exogenous reporter genes may have fewer off-target  
162 effects.

163 To compare the IGE system with artificial microRNAs (amiRNA) (Fig. 1b), a popular gene  
164 knockdown strategy<sup>28,29</sup>, we generated two amiRNAs targeting *PLT2* in *gPLT2-3xYFP; plt1,2*.  
165 Induction of *amiPLT2-1* by *ip35S* or *ipWOX5* led to a reduction of YFP in a broader domain than  
166 with *PLT2*-IGE (Supplementary Fig. 12a), indicating that IGE is more specific. This is likely due to  
167 cell-to-cell movement of amiRNA, consistent with the findings that several microRNAs can move<sup>4</sup>.  
168 Additionally, the IGE-caused phenotype tended to be stronger. After a 3-day induction of  
169 *ip35S:amiPLT2-1*, the YFP signal was decreased but still visible, and the RM remained  
170 undifferentiated after 10 days of induction (Supplementary Fig. 12a and Supplementary Table 1).  
171 Likewise, no QC differentiation was observed in *ipWOX5:amiPLT2-1* lines (Supplementary Fig.  
172 12a). The RM of *amiGORBR* showed an overproliferation phenotype, but it was not as severe as in

173 RBR-IGE lines (Supplementary Fig. 12b). To investigate the effect of RBR downregulation in other  
174 tissues, we analyzed the root vascular tissue during secondary growth. While *amiGORBR* failed to  
175 show any defects in secondary tissue, RBR-IGE caused excessive cell divisions in the phloem and  
176 periderm (Supplementary Fig. 12b), indicating a conserved role for RBR in limiting cell divisions  
177 in different tissues. Interestingly, the proliferating clones were interspaced with slowly proliferating  
178 WT clones, which further confirms the cell-autonomous function of RBR.

179 In conclusion, we show that the IGE system can be used to disrupt target genes efficiently and  
180 precisely. Through spatiotemporal control of Cas9p expression, the system is well-suited to trace  
181 early molecular and cellular changes before visible phenotypes appear. Since the estrogen inducible  
182 system has been applied in various organs and plant species<sup>12,30,31</sup>, we expect the IGE system to be  
183 broadly applicable for plant molecular biology. By using different Cas9 variants, the system can be  
184 readily repurposed for base editing or transcriptional regulation.

## 185 **METHODS**

### 186 **Plant material and cloning**

187 To generate the *p221z-Cas9p-t35s* entry vector, first, *Cas9p* with two flanking nuclear localized  
188 signal (*NLS*) coding sequence and a *t35* terminator were amplified from vector  
189 *pYLCRISRPCas9P35S-B*<sup>10</sup> with chimeric primers which contained the *attB1/attB2* adaptor at the 5'  
190 end and a 3' end complementary to *NLS* and *t35s*, respectively. The resultant PCR fragment was  
191 gel-purified and then recombined with *pDONR 221* following the instructions of the Gateway BP  
192 Clonase II Enzyme mix (Invitrogen).

193 Site-directed mutations were introduced to two nuclease domains of Cas9p, RuvC1 and HNH  
194 (D10A, H840A)<sup>32</sup>, respectively, to generate dCas9. To achieve this, a partial *Cas9p* fragment (61-  
195 2582, starting from ATG) was amplified with primers containing the desired mutations. The  
196 purified PCR fragment was then used as a mega-primer to amplify *p221z-Cas9p-t35s*. The resulting



197 PCR product was digested by methylation-specific endonuclease Dpn I to remove the parental DNA  
198 template before transformation into competent *E.coli* DH5 $\alpha$  cells. The presence of mutations in  
199 *p221z-dCas9p-t35s* (Addgene ID: 118387) was verified by Sanger sequencing.

200 To insert the *tagRFP* sequence between *Cas9p* and the 3' end of the *NLS* encoding sequence located  
201 in *p221z-Cas9p-t35s*, *tagRFP* was first amplified from the entry vector *p2R3a-tagRFP-OcsT*<sup>11</sup> with  
202 chimeric primers consisting of a 3' end of *tagRFP*-specific oligonucleotides and a 5' end of  
203 *Cas9p/NLS*-specific oligonucleotides complementary to the flanking sequence at the insertion point.  
204 The purified PCR fragment was then used as mega-primer in the subsequent Omega PCR step<sup>33</sup>,  
205 which used *p221z-Cas9p-t35s* as the template. The PCR product was treated with Dpn I before  
206 transformation into competent *E.coli* DH5 $\alpha$  cells. The insertion of *tagRFP* was verified by both  
207 enzyme digestion and Sanger sequencing.

208 To facilitate ligation of the sgRNA expression cassette (*pAtU3/6-sgRNA*) into a Gateway entry  
209 vector, the negative selection marker, a *ccdB* expression cassette flanked by two *Bsa I* sites, was  
210 amplified from *pYLCRISPRCas9P35S-B*<sup>10</sup> with primers containing *attB2/attB3* adaptors. After a BP  
211 reaction with *pDONR P2R-P3z*, the reaction mixture was transformed into the *ccdB*-tolerant *E.coli*  
212 strain DB3.1. Colony PCR was performed to screen for positive colonies which had been  
213 transformed with recombined plasmids but not the empty *pDONR-P2R-P3z*. The presence of the  
214 *p2R3z-Bsa I-ccdB-Bsa I* entry vector was then further confirmed by enzyme digestion and Sanger  
215 sequencing.

216 The sgRNA expression cassettes were obtained as previously described<sup>10</sup>. Briefly, the first round of  
217 PCR amplified *AtU3/6* promoters from template vectors, *pYLSgRNA-AtU3b* (Addgene ID: 66198),  
218 *pYLSgRNA-AtU3d* (Addgene ID: 66200), *pYLSgRNA-AtU6-1* (Addgene ID: 66202) or *pYLSgRNA-*  
219 *AtU6-29* (Addgene ID: 66203), using a common forward primer, *U-F*, and reverse chimeric primer  
220 *U3/6 T#*- which contains an *AtU3/6*-specific sequence at the 3' end and a target sequence at the 5'  
221 end. All sgRNA scaffolds were amplified from *pYLSgRNA-AtU3b* with a common reverse primer,

222 *gR-R*, and chimeric forward primer *gRT #+*, which includes the sgRNA specific sequence at the 3'  
223 end and the target sequence at the 5' end. In the second round of PCR, purified first-round PCR  
224 products were used as templates for overlapping PCR with *Bsa I*-containing primers *Pps/Pgs* as  
225 primer pairs. In this study, four sgRNAs (sgRNA1-sgRNA4) transcribed under promoters *AtU3b*,  
226 *AtU3d*, *AtU6-1*, and *AtU6-29*, respectively, were used to target genes of interest. For each target  
227 gene, four relatively equally distributed target sites were manually selected by following rules  
228 described previously<sup>10</sup>. Different sgRNA expression cassettes were cloned into the *p2R3z-Bsa I-*  
229 *ccdB-Bsa I* entry vector by one-step Golden Gate cloning. Golden gate cloning was performed with  
230 120ng *p2R3z-Bsa I-ccdB-Bsa I*, 90 ng purified PCR product of each sgRNA expression cassette,  
231 1.5μl 10x fast digestion buffer of *Bsa I*, 1.5μl *Bsa I* enzyme (15U), 1.5μl 10mM ATP, 4μl T4 DNA  
232 ligase (20U), and H<sub>2</sub>O to make up 15μl. The reaction mixture was incubated at 37 °C for 4-6h  
233 before *E. coli* transformation. Selection of positive transformants was performed as described  
234 above.

235 To generate the *p221z-AtMIR390a* entry vector (Fig. 1b), a BP reaction was performed with  
236 *pDONR 221* and *pMDC123SB-AtMIR390a-B/c*<sup>28</sup> (Addgene ID: 51775). *pMDC123SB-AtMIR390a-*  
237 *B/c* contains *AtMIR390a* 5' end and *AtMIR390a* 3' end which were split by *Bsa I*-flanking *ccdB*  
238 expression modules. After transforming DB3.1, positive colonies were screened by colony PCR  
239 followed by enzyme digestion and sequencing. Two artificial microRNA against *PLT2* (*amiPLT2-1*  
240 and *amiPLT2-2*) were designed using <http://p-sams.carringtonlab.org/>. Annealed *amiPLT2* was  
241 ligated into *p221z-AtMIR390a* by a one-step reaction as previously described<sup>28</sup>.

242 Tandem arrayed tRNA-sgRNA units have been exploited for multiplex genome editing by using the  
243 endogenous tRNA processing machinery<sup>34</sup>, which precisely cuts tRNA precursors at both ends and  
244 releases free sgRNA after transcription. This strategy has been applied in a variety of plant  
245 species<sup>34,35</sup>. However, to date there are few reports of its application in *Arabidopsis*. We therefore  
246 investigated its feasibility in *Arabidopsis* genome editing and meanwhile tested its compatibility

247 with our IGE system. To facilitate target sequence ligation, we first constructed a *p2R3z-AtU3b-*  
248 *tRNA-ccdB-sgRNA* entry vector (Fig. 1b). AtU3b, tRNA-1, tRNA-2 (tRNA was amplified in two  
249 separate fragments), the *ccdB* expression cassette (flanked by Bsa I), and the sgRNA scaffold were  
250 amplified with the indicated primer pairs. Both ends of each fragment contained primer-introduced  
251 sequences overlapping with the desired flanking fragments. In the overlapping PCR step, *attB2-*  
252 *AtU3b-F* and *attB3-sgRNA-R* were used as a primer pair to assemble these five purified PCR  
253 fragments, which were mixed as templates. Cloning this fused fragment into *pDONR P2R-P3z* was  
254 conducted as described above. To clone the first target sequence of *PLT2* into *p2R3z-AtU3b-tRNA-*  
255 *ccdB-sgRNA*, two annealed primers with 4-nucleotide overhangs at the 5' ends and 20-nucleotide  
256 complementary target sequences were ligated into the entry vector in a one-step reaction as  
257 described previously<sup>28</sup>. In the *Arabidopsis* RM, we observed a decrease of the YFP signal in the  
258 region where the inducible promoter was active in most independent lines after a 1-day induction  
259 and finally a fully differentiated RM after a 10-day induction (Supplementary Fig. 3;  
260 Supplementary table 1), indicating that sgRNA against *PLT2* was disassociated from tRNA  
261 processing and guiding Cas9p to cleave *PLT2*. It has recently been reported that efficient genome  
262 editing could be achieved by fusing tRNA to a mutant sgRNA scaffold but not the wild type sgRNA  
263 scaffold in *Arabidopsis*<sup>36</sup>. However, in our hands wild type sgRNA scaffold and tRNA fusion  
264 worked well. We reasoned that the sgRNA promoter, Cas9 variant, sgRNA scaffold, target loci, and  
265 the tissue to be edited may all affect tRNA-sgRNA-mediated editing performance in *Arabidopsis*.  
266 Therefore a future comprehensive study of these variables may improve the utility of the tRNA  
267 processing system in *Arabidopsis*.

268 The red seed coat vector *pFRm43GW* (Addgene ID: 133748) was generated by modifying the  
269 *pHm43GW* destination vector<sup>13</sup>, which was obtained from VIB (<https://gateway.psb.ugent.be/>). The  
270 *pHm43GW* vector was digested with PaeI (SphI) (ThermoFisher Scientific) to remove the  
271 hygromycin cassette. Using an In-Fusion HD Cloning (TaKaRa) kit, two fragments were cloned

272 into the digested vector. The first fragment contained a *ccdB* cassette and recombination sites for  
273 MultiSite Gateway cloning, and it was amplified from *pHm43GW* using  
274 GAACCCTGTGGTTGGCATGCACATACAAATGGACGAACGGATAAA as a forward primer  
275 and ATACCTACATACACTTGAAGGGTACCCGGGGATCCTCTAGAGGG as a reverse primer.  
276 The second fragment contained the FastRed module, consisting of the *OLE1* promoter followed by  
277 *OLE1-tagRFP*, which was amplified from *pFAST-R01*<sup>37</sup> using  
278 CTTCAAGTGTATGTAGGTATAGTAACATG as a forward primer and  
279 CGAATTGAATTATCAGCTTGCATGCAGGGTACCATCGTTCAAACATTTGGCAAT as a  
280 reverse primer.

281 We also provide another non-destructive fluorescent screening vector, the green seed coat vector  
282 *pFG7m34GW* (Addgene ID: 133747). It was generated by cloning the FastGreen module into the  
283 *pP7m34GW* vector<sup>13</sup>, which was obtained from VIB (<https://gateway.psb.ugent.be/>). The  
284 *pP7m34GW* vector was digested with SacI (ThermoFisher Scientific). Three fragments were cloned  
285 into the digested *pP7m34GW*. The first fragment contained the *OLE1* promoter followed by the  
286 *OLE1* genomic sequence and was amplified from *pFRm43GW* using  
287 CCATATGGGAGAGCTCCTTCAAGTGTATGTAGGTATAGT as a forward primer and  
288 GCCCTTGCTCACCATAGTAGTGTGCTGGCCACCACGAG as a reverse primer; the second  
289 fragment contained the *EGFP* encoding sequence and was amplified from the *pBGWFS7* vector<sup>13</sup>  
290 using ATGGTGAGCAAGGGCGAGGAGCTGT as a forward primer and  
291 ATCTATGTTACTAGATCACTTGTACAGCTCGTCCATGCC as a reverse primer; the third  
292 fragment contained the *nosT* terminator sequence and was amplified from the *p1R4-ML:XVE*  
293 vector<sup>11</sup> using TCTAGTAACATAGATGACACCGCGCG as a forward primer and  
294 TTAACGCCGAATTGAATTCGAGCTCCATCGTTCAAACAT as a reverse primer. All three  
295 fragments were combined together with the digested vector using In-Fusion HD Cloning.

296 The five inducible promoters (*p1R4-p35S:XVE*, *p1R4-pSCR:XVE*, *p1R4-pWER:XVE*, *p1R4-*  
297 *pWOL:XVE*) were created earlier<sup>11</sup>. To construct the binary vector, a MultiSite Gateway LR reaction  
298 was performed with the inducible promoters in the 1<sup>st</sup> box, *Cas9p*, *dCas9p*, *Cas9p-tagRFP* or  
299 *amiPLT2* in the 2<sup>nd</sup> box, the sgRNA expression cassette or *nosT* terminator in the 3<sup>rd</sup> box and  
300 *pBm43GW* (PPT (phosphinotricin) selection) or *pFRm43GW* (seed coat RFP selection) as the  
301 destination vectors. All constructs generated in this study are listed in Supplementary table 3.

302 *PLT2*-targeting constructs were dipped into the *gPLT2:3xYFP,plt1,2* background<sup>16</sup>. For *RBR*-  
303 targeting constructs, the dipping background was segregating *pRBR:RBR-YFP(+,-);*  
304 *35S:amiGORBR(+,+)*<sup>19</sup>. The IGE construct targeting *GNOM* was transformed into both the *GN-*  
305 *GFP*<sup>20</sup> and *PINI-GFP*<sup>25</sup> backgrounds. With the exception of the construct transformed into the GN-  
306 GFP background, in which the GFP signal was weak, all T1 lines were prescreened under a  
307 fluorescence-binocular microscope to identify those with leaky inducible promoter or in which the  
308 root tip had been damaged during selection. Only lines with YFP/GFP signal in root tip were used  
309 for further experiments. The *PLT2* and *RBR*-based backgrounds were also used for *YFP*-targeting  
310 construct transformation. The *RBR*-targeting construct *ip35S>>Cas9p-RBR* was also dipped into  
311 the *Col-0* background. All experiments were conducted using T1 plants unless stated otherwise.  
312 Each experiment has been repeated at least three times, except the RM differentiation  
313 characterization in Supplementary Table 1, which was repeated twice.

#### 314 **Plant growth and chemical treatments**

315 All seeds were surface-sterilized with 20% chlorine for 1 min, followed by a 1 min incubation in  
316 70% ethanol and two rinses in H<sub>2</sub>O. The sterilized seeds were kept at 4°C for two days before  
317 plating on half strength Murashige and Skoog growth medium (½ GM) plates with/without  
318 selection antibiotics. The plates were vertically positioned in a growth chamber at 22 °C in long day  
319 conditions. PPT selection was conducted by growing sterilized seeds on ½ GM plates containing 20  
320 µg/ml PPT for 4 days, then transferring them to PPT-free ½ GM plates for another 2 days before

321 treatment. The trans-pFRm43GW-based seeds were screened under a fluorescence binocular using  
322 DSRed filter (Supplementary Fig. 1b), and the sterilized seeds were directly grown on ½ GM plates  
323 for 6 days before treatment. 17-β-estradiol (17-β, Sigma) was dissolved in dimethyl sulfoxide  
324 (DMSO, Sigma) to make 10 mM stock solution (stored at -20°C) and a 5 μM working concentration  
325 was used. An equal volume of DMSO was used as a mock treatment.

### 326 **Microtome sectioning and histological staining**

327 Transverse plastic sections were cut from *ip35S>>Cas9p-RBR* (in *Col-0* background) roots which  
328 were germinated on estradiol plates for 20 days, as well as *Col-0* and *35S:amiGORBR* roots that  
329 were grown on ½ GM plates for 20 days. Sections from 5 mm below the root–hypocotyl junction  
330 point were used for analysis. Sections were stained in 0.05% (w/v) ruthenium red solution (Fluka  
331 Biochemika) for 5 seconds before microscopy analysis. For root samples from *ipWOX5>>Cas9p-*  
332 *tagRFP-PLT2*, *ipWOX5>>Cas9p-tagRFP-YFP* and *ipWOX5>>amiPLT2-1*, after 3 days of mock or  
333 17-β treatment, a serial longitudinal section of 5 μm thickness was cut from the root tips. To  
334 observe the QC differentiation state, the longitudinal sections were stained in 1g/ml lugol solution  
335 (Sigma) for 12 seconds before observation under a microscope. The sectioning methodology has  
336 been previously described<sup>38</sup>.

### 337 **Microscopy and image processing**

338 All of the cross sections and longitudinal sections were visualized using a Leica 2500 microscope.  
339 All fluorescent images were taken with a Leica TCS SP5 II Confocal microscope. Root samples  
340 used for cell death detection were stained in 10 μg/mL propidium iodide for 10 mins then rinsed  
341 twice in water before imaging. For other samples used for fluorescence observation, a ClearSee  
342 protocol<sup>39</sup> was used with slight modifications. Roots were first fixed in 4% paraformaldehyde  
343 (dissolved in 1xPBS, PH 7.2) for at least one hour with vacuuming, then washed twice in 1x PBS  
344 and transferred to ClearSee solution. Samples were incubated in ClearSee solution for at least 24h.

345 Before imaging, 0.1% calcofluor white dissolved in ClearSee was used for one hour with  
346 vacuuming to stain cell walls. This was followed by washing the samples in ClearSee solution for at  
347 least 30 mins with shaking. During the washing, the ClearSee solution was changed every 15 mins.  
348 Confocal settings were kept the same between mock and induction in each experiment. All confocal  
349 images were acquired in sequential scanning mode. Images were sometimes rotated using  
350 Photoshop and the resulting empty corners were filled with a black background. All images were  
351 cropped and organized in Microsoft PowerPoint. The brightness of the calcofluor signal was  
352 sometimes adjusted differently between the mock and induction for better cell wall visualization.

### 353 **ACKNOWLEDGEMENT**

354 We thank B. Scheres (Wageningen University, the Netherlands) and N. Geldner (University of  
355 Lausanne, Switzerland) for sharing published materials; N. Geldner for providing support for R.U.;  
356 and S. el-Showk for proofreading of this manuscript. This work was supported by the Academy of  
357 Finland (grants #316544, #266431, #307335), University of Helsinki HiLIFE fellowship (X.W.,  
358 L.Y., A.P.M.) and European Molecular Biology Organisation (EMBO ALTF 1046-2015 to R.U.).  
359 X.W. is also supported by a grant from the Chinese Scholarship Council (CSC).

### 360 **CONTRIBUTIONS**

361 X.W. and A.P.M. designed the experiments. X.W. conducted all experiments, except L.Y. carried out  
362 the analysis for Supplementary Table 1. R.U. generated and tested the new destination vectors. X.W.  
363 and A.P.M. analyzed the results and wrote the manuscript, with input from all co-authors.

### 364 **COMPETING INTERESTS STATEMENT**

365 The authors declare no competing financial interests.

### 366 **FIGURE LEGENDS**

367 **Figure 1: Engineering the IGE system for conditional genome editing**



368 **a**, Cloning steps for IGE construct generation. Fusions of the sgRNA expression cassette (*pAtU3/6-*  
369 *sgRNA*) were constructed by two PCR steps and were subsequently cloned into the *p2R3z-Bsa I-*  
370 *ccdB-Bsa I* entry vector by Golden Gate cloning. The binary IGE construct was then recombined by  
371 a MultiSite Gateway LR reaction. **b**, Schematics of two other entry vectors generated in this study.  
372 Entry vector *p221z-AtMIR390a*, in which *AtMIR390a* is split by a *Bsa I*-flanking-*ccdB* cassette,  
373 was utilized for inducible gene knockdown. Entry vector *p2R3z-AtU3b-tRNA-ccdB-gRNA* was  
374 generated to exploit the endogenous tRNA processing system. Two annealed overlapping target  
375 sequences with overhangs can be directly ligated into *Bsa I*-linearized *p2R3z-AtU3b-tRNA-ccdB-*  
376 *gRNA*. Red numbers in brackets are the Addgene numbers of vectors created in this study. **c**, The  
377 YFP signal in the RM of 7 day-old *gPLT2-3xYFP;plt1,2*. **d**, dCas9p does not decrease PLT2-3xYFP  
378 expression. **e**, Cas9p-mediated *PLT2* editing resulted in a gradual loss of YFP and eventually full  
379 differentiation of the RM. The numbers are the frequency of the observed phenotypes in  
380 independent T1 samples. Cell walls are visualized by calcofluor. Scale bar, 50  $\mu$ m.

## 381 **Figure 2: The IGE system enables efficient cell-type-specific genome editing**

382 **a**, A one-day induction is sufficient to induce efficient cell-type specific *PLT2* editing. In rare  
383 occasions, we observed overlapping Cas9p-tagRFP and PLT2-3xYFP expression (white arrowhead).  
384 **b**, *PLT2* is cell-autonomously required for QC and stem cell maintenance. QC cells (red  
385 arrowheads) as well as endodermal and epidermal cells (white arrows) showed premature  
386 differentiation or cell expansion after 3 days of induction. QC differentiation is accompanied by  
387 shift of *ipWOX5* expression towards the provascular cells. Removal of *PLT2* from the *ipWER*  
388 expression domain also resulted in fewer LRC layers (white arrowhead) and ectopically decreased  
389 the PLT2-3xYFP expression. Cas9p-tagRFP expression in the LRC and epidermis was frequently  
390 undetectable. **c**, A one-day induction is sufficient to induce efficient cell-type specific *RBR* editing.  
391 Without induction, the QC frequently shows cell divisions, probably due to the heterogeneity of the  
392 complementing RBR-YFP. **d**, RBR cell-autonomously prevents QC and stem cell division. The



393 endodermis, QC and LRC exhibited overproliferation after 3 days of induction. White arrowheads  
394 indicate rotated cell division planes in the endodermis. QC regions are marked by brackets in **c** and  
395 **d**. Cell walls are highlighted by calcofluor. The numbers represent the frequency of the observed  
396 phenotypes in independent T1 samples. Scale bars, 50  $\mu\text{m}$ .

397

398

### 399 SUPPLEMENTARY FIGURE LEGENDS

#### 400 **Supplementary Figure 1 Non-destructive screening markers facilitate identification of** 401 **transformed seeds.**

402 **(a)** Non-destructive fluorescent screening destination vectors generated in this study. **(b)** Examples  
403 of trans-pFRm43GW seeds screened under the fluorescence-binocular in the T1 (left) and T2 (right)  
404 generations.

#### 405 **Supplementary Figure 2 PCR genotyping of *PLT2* deletions.**

406 **(a)** Tandem arrayed sgRNA expression cassettes. **(b)** The genomic structure of *PLT2*. Boxes  
407 indicate exons. Orange bars represent target sites in *PLT2*. Black arrows represent relative positions  
408 of the forward and reverse primers. **(c)** PCR detection of *PLT2* deletion in *ip35S>>Cas9p-PLT2*;  
409 *gPLT2-3xYFP;plt1,2* T1 seedlings after 3 days of treatment (in 6 day-old plants). Pooled DNA was  
410 isolated from 2cm root segments below the hypocotyl of 10 seedlings. Three primer pairs were  
411 used. There were no detectable truncated bands in 7-day old *gPLT2 3xYFP;plt1,2*, while weak  
412 truncated bands were detected in mock treated seedlings (white arrowhead), probably due to weak  
413 leakiness of *ip35S* in certain roots or cells. Note that although four sgRNAs were used to target  
414 *PLT2*, only one truncated band was detected with each primer pair. **(d)** Sequencing of truncated  
415 bands from primer pair F-R3 confirmed deletion between the 1<sup>st</sup> and 4<sup>th</sup> *PLT2* target sites (letters in

416 red represent protospacer adjacent motif, PAM). To determine the deletion types, the truncated band  
417 was not directly used for sequencing but cloned into *pDONR 221*. Two deletion types were found in  
418 4 sequenced recombinant vectors.

419 **Supplementary Figure 3 sgRNA promoter identity affects editing efficiency in *Arabidopsis***  
420 **roots.**

421 For each construct, the indicated sgRNA promoter was used to drive transcription of sgRNA1,  
422 while *ip35S* was used to guide *Cas9p* transcription. *AtU3b* and *AtU6-29* showed the best editing  
423 efficiency in T1 seedlings after one day of induction. Transcription of tRNA together with sgRNA1  
424 under the *AtU3b* promoter also resulted in efficient *PLT2* editing. WT is the 7-day old *gPLT2-*  
425 *3xYFP; plt1,2*. White dotted lines mark the RM outlines. Cell walls are highlighted by calcofluor.  
426 Numbers indicate the frequency of similar results in the independent T1 samples analyzed. Scale  
427 bar, 50  $\mu$ m.

428 **Supplementary Figure 4 IGE-mediated genome editing correlates with Cas9 expression.**

429 After one day of induction, IGE performance on *PLT2* editing under different inducible promoters  
430 was classified into two categories. In the mild category, Cas9p-tagRFP expression tends to be weak  
431 and narrow, resulting in narrow domains of moderately decreased YFP signal. In the strong  
432 category, Cas9p-tagRFP expression was strong and broad, with strongly and broadly reduced YFP  
433 fluorescence. In the uppermost panel, Cas9p was used without a tag. White dotted lines mark the  
434 RM outlines. Cell walls are visualized by calcofluor. Numbers indicate the frequency of similar  
435 results in the T1 samples analyzed. Scale bars, 50  $\mu$ m.

436 **Supplementary Figure 5 IGE system enables real time observation of genome editing.**

437 To monitor *PLT2* editing dynamics, a time-course 17- $\beta$  induction was conducted to *ipWER* >>  
438 *Cas9p-tagRFP-PLT2* in *gPLT2-3xYFP; plt1,2* (T2 generation, #1). The reduction of *PLT2-3xYFP*  
439 expression was first detected after 12 hours of induction and became obvious with 16 hours of

440 induction. The editing activity was gradually spread inwards, likely due to the radial diffusion of  
441 17- $\beta$  within *ipWER* domain. White dotted lines mark the RM outlines. Cell walls are visualized by  
442 calcofluor. Numbers indicate the frequency of observed phenotype within given induction duration.  
443 Scale bar, 50  $\mu$ m.

444 **Supplementary Figure 6 The capacity of conditional genome editing by IGE system is**  
445 **inherited.**

446 For each construct, two independent transgenic T2 lines were randomly selected and checked.  
447 Representative images are shown. Note that the second *ipWOX5>>Cas9p-tagRFP-PLT2* line was  
448 leaky: roots displayed a similar phenotype with/without induction. Cell walls are marked by  
449 calcofluor. Numbers represent the frequency of the observed phenotype in analyzed T2 samples.  
450 Scale bar, 50  $\mu$ m.

451 **Supplementary Figure 7 RBR functions cell-autonomously in the RM.**

452 **(a)** A three-day mock treatment of *ip35S>>Cas9p-RBR* in *RBR-YFP; amiGORBR*. **(b)** A one-day  
453 induction caused a reduced RBR-YFP signal mainly in the root cap region without an obvious  
454 phenotype. **(c)** Inducing *RBR* editing with *ip35S* typically led to LRC overproliferation (white  
455 arrows) without affecting the YFP signal in other domains after a 3-day induction. In some cases,  
456 both wild type cells and RBR-knockout cells were seen on the same root (left in **c**). Cell walls are  
457 visualized by calcofluor. Numbers indicate the frequency of the observed phenotype in independent  
458 T1 samples. Scale bar, 50  $\mu$ m.

459 **Supplementary Figure 8 Post-embryonically inducing *GNOM* editing recapitulates the**  
460 **phenotypes of the *gnom* mutant.**

461 **(a)** Plants with *ipWOL>>Cas9p-tagRFP-GNOM; PIN1-GFP* ten days after germination on mock or  
462 17- $\beta$  plates. Inducing *GNOM* editing led to shorter roots, agravitropic growth and decreased lateral  
463 root (LR) numbers. Adventitious roots from the hypocotyl were frequently found, but these roots

464 were not counted in LR quantification. For each independent root, LR number and root length is  
465 quantified in (b). Scale bar, 1 cm.

466 **Supplementary Figure 9 GNOM is required for PIN1 polarity and expression.**

467 (a) *GNOM* expression disappeared from the vasculature after a 6-day induction of IGE targeting  
468 *GNOM*. Due to the weak GFP signal, only roots showing a clear loss of GFP signal were included  
469 in quantification. (b) A three-day induction of *ipWOL>> Cas9p-tagRFP-GNOM; PIN1-GFP*  
470 resulted in loss of polarity and decreased expression of PIN1-GFP in the endodermis (en), pericycle  
471 (p) and stele (s) (white arrows). Right panels are magnified images of the regions marked with a red  
472 box in the left panels. Cell walls are marked by calcofluor. Numbers indicate the frequency of the  
473 observed phenotype in independent T1 samples analyzed. Scale bar in right panels of a, 25  $\mu\text{m}$ ;  
474 others, 50  $\mu\text{m}$ .

475 **Supplementary Figure 10 Cas9p-mediated genome editing in proximal stem cells induces cell**  
476 **death.**

477 (a) Stem cell death surrounding the QC was observed after one day of *ip35S>>Cas9p-PLT2*  
478 induction. Based on cell types, the cell death response is classified into three categories: provascular  
479 cell death, LRC/epidermis initial cell death and columella initial cell death. Samples were counted  
480 twice if they had cell death in different domains. (b) Cell death of provascular cells and early  
481 descendants was induced after one day of induction of *ipWOL>>Cas9p-tagRFP-*  
482 *PLT2/RBR/GNOM*. Cell walls are highlighted by propidium iodide (PI). Under PI detection  
483 settings, Cas9p-tagRFP is also visible. Numbers indicate the frequency of the observed phenotype  
484 in independent T1 samples analyzed. Scale bars, 50  $\mu\text{m}$ .

485 **Supplementary Figure 11 A single IGE construct targeting a gene encoding a fluorescent**  
486 **reporter has the potential to disrupt different transgene targets.**

487 (a) Editing *YFP* instead of *PLT2* in the *ipWER* expression region caused changes similar to direct  
488 *PLT2* editing. The RM had fewer LRC layers (white arrowheads), as well as premature expansion  
489 of epidermal cells and a broad, faint YFP signal. The Cas9p-tagRFP signal is frequently invisible.  
490 (b) Editing *YFP* led to QC (black arrow) differentiation at a lower frequency. (c) Targeting the *YFP*  
491 of RBR-YFP in the LRC led to LRC overproliferation, similar to editing RBR. However, the YFP  
492 signal outside *ipWER* expression region was also hampered by an unknown mechanism, unlike  
493 when editing *RBR*. White arrows mark the neighboring cell walls in a and c. The same construct  
494 was used in a and c. Cell walls are highlighted by calcofluor. Numbers indicate the frequency of the  
495 observed phenotype in independent T1 samples analyzed. Scale bars, 50  $\mu\text{m}$ .

#### 496 **Supplementary Figure 12 Comparison of IGE system with inducible amiRNA.**

497 (a) IGE-*PLT2* displays more specific and stronger *PLT2-YFP* downregulation than ami*PLT2*. After  
498 a one-day induction, *ip35S>>amiPLT2-1; gPLT2-3xYFP;plt1,2* and *ipWOX5>>amiPLT2-1;*  
499 *gPLT2-3xYFP;plt1,2* showed a broader reduction of the YFP signal, particularly in the bracketed  
500 regions where no inducible promoter activity was found. Conversely, induced *PLT2* editing caused  
501 very local loss of the YFP signal. After a three-day induction, the YFP signal is still visible in most  
502 of *ip35S>>amiPLT2-1; gPLT2-3xYFP; plt1,2* transformants but not in *ip35S>>Cas9p-PLT2;*  
503 *gPLT2-3xYFP;plt1,2* transformants. There was no QC differentiation in *ipWOX5>>amiPLT2-1;*  
504 *gPLT2-3xYFP; plt1,2* roots. WT here means 7-day old *gPLT2-3xYFP; plt1,2*. White arrows mark  
505 the QC. (b) Comparison of the RM and root secondary growth of *Col-0*, *35S:amiGORBR* and  
506 *ip35S>>Cas9p-RBR*. Inducing *RBR* editing (germination and six days of growth on 17- $\beta$  plates)  
507 resulted in more excessive cell divisions in the LRC than was seen in *amiGORBR* roots  
508 (germination and six days of growth on 17- $\beta$  free plates). Furthermore, *RBR* editing caused cell  
509 overproliferation in secondary tissues such as phloem (ph) cells and the periderm (pe), which was  
510 not observed in *amiGORBR* roots. The knockout (ko) sectors (green dotted line) were frequently  
511 accompanied by WT sectors (red dotted line), which can be regarded as an internal control. Cell

512 walls are marked by calcofluor. Numbers indicate the frequency of observed phenotype in  
513 independent samples analyzed. Scale bars, 50  $\mu$ m.

514 **Supplementary Table 1 Quantification of fully differentiated RM after 10 days induction.**

515 **Supplementary Table 2 Primer list in this study.**

516 Underlined sequences indicate Gateway adaptors. Sequence in red represent the target sequence in  
517 the gene.

518 **Supplementary Table 3 Constructs list in this study.**

## 519 REFERENCES

- 520 1. Candela, H., Perez-Perez, J. M. & Micol, J. L. Uncovering the post-embryonic functions of  
521 gametophytic- and embryonic-lethal genes. *Trends Plant Sci.* **16**, 336-345 (2011).
- 522 2. Borghi, L. *et al.* Arabidopsis RETINOBLASTOMA-RELATED is required for stem cell  
523 maintenance, cell differentiation, and lateral organ production. *Plant Cell* **22**, 1792-1811 (2010).
- 524 3. Guo, J., Wei, J., Xu, J. & Sun, M. X. Inducible knock-down of GNOM during root formation  
525 reveals tissue-specific response to auxin transport and its modulation of local auxin biosynthesis. *J.*  
526 *Exp. Bot.* **65**, 1165-1179 (2014).
- 527 4. Liu, L. & Chen, X. Intercellular and systemic trafficking of RNAs in plants. *Nat. Plants* **4**, 869-  
528 878 (2018).
- 529 5. Heidstra, R., Welch, D. & Scheres, B. Mosaic analyses using marked activation and deletion  
530 clones dissect Arabidopsis SCARECROW action in asymmetric cell division. *Genes Dev.* **18**, 1964-  
531 1969 (2004).
- 532 6. Wachsman, G., Heidstra, R. & Scheres, B. Distinct cell-autonomous functions of  
533 RETINOBLASTOMA-RELATED in Arabidopsis stem cells revealed by the Brother of Brainbow  
534 clonal analysis system. *Plant Cell* **23**, 2581-2591 (2011).
- 535 7. Wildwater, M. *et al.* The RETINOBLASTOMA-RELATED gene regulates stem cell maintenance  
536 in Arabidopsis roots. *Cell* **123**, 1337-1349 (2005).
- 537 8. Mao, Y. F., Botella, J. R., Liu, Y. G. & Zhu, J. K. Gene editing in plants: progress and challenges.  
538 *Natl. Sci. Rev.* **6**, 421-437 (2019).
- 539 9. Decaestecker, W. *et al.* CRISPR-TSKO facilitates efficient cell type-, tissue-, or organ-specific  
540 mutagenesis in Arabidopsis. *bioRxiv*, 474981 (2018).
- 541 10. Ma, X. *et al.* A robust CRISPR/Cas9 System for Convenient, High-Efficiency Multiplex  
542 Genome Editing in Monocot and Dicot plants. *Mol. Plant* **8**, 1274-1284 (2015).
- 543 11. Siligato, R. *et al.* Multisite Gateway-Compatible Cell Type-Specific Gene-Inducible System for  
544 Plants. *Plant Physiol.* **170**, 627-641 (2016).
- 545 12. Zuo, J., Niu, Q. W. & Chua, N. H. Technical advance: An estrogen receptor-based transactivator  
546 XVE mediates highly inducible gene expression in transgenic plants. *Plant J.* **24**, 265-273 (2000).
- 547 13. Karimi, M., Inze, D. & Depicker, A. GATEWAY vectors for Agrobacterium-mediated plant  
548 transformation. *Trends Plant Sci.* **7**, 193-195 (2002).
- 549 14. Aida, M. *et al.* The PHETHORA genes mediate patterning of the Arabidopsis root stem cell  
550 niche. *Cell* **119**, 109-120 (2004).

- 551 15. Galinha, C. *et al.* PLETHORA proteins as dose-dependent master regulators of Arabidopsis root  
552 development. *Nature* **449**, 1053-1057 (2007).
- 553 16. Mähönen, A. P. *et al.* PLETHORA gradient formation mechanism separates auxin responses.  
554 *Nature* **515**, 125-129 (2014).
- 555 17. Vandenberg, C., Willemsen, V., Hage, W., Weisbeek, P. & Scheres, B. Cell fate in the  
556 Arabidopsis root-meristem determined by directional signaling. *Nature* **378**, 62-65 (1995).
- 557 18. Ebel, C., Mariconti, L. & Gruijsem, W. Plant retinoblastoma homologues control nuclear  
558 proliferation in the female gametophyte. *Nature* **429**, 776-780 (2004).
- 559 19. Cruz-Ramirez, A. *et al.* A SCARECROW-RETINOBLASTOMA protein network controls  
560 protective quiescence in the Arabidopsis root stem cell organizer. *Plos Biol.* **11**, e1001724 (2013).
- 561 20. Geldner, N. *et al.* The Arabidopsis GNOM ARF-GEF mediates endosomal recycling, auxin  
562 transport, and auxin-dependent plant growth. *Cell* **112**, 219-230 (2003).
- 563 21. Kleine-Vehn, J. *et al.* ARF-GEF-dependent transcytosis and polar delivery of PIN auxin carriers  
564 in Arabidopsis. *Curr. Biol.* **18**, 526-531 (2008).
- 565 22. Steinmann, T. *et al.* Coordinated polar localization of auxin efflux carrier PIN1 by GNOM ARF  
566 GEF. *Science* **286**, 316-318 (1999).
- 567 23. Geldner, N. *et al.* Partial loss-of-function alleles reveal a role for GNOM in auxin transport-  
568 related, post-embryonic development of Arabidopsis. *Development* **131**, 389-400 (2004).
- 569 24. Shevell, D. E. *et al.* Emb30 is essential for normal-cell division, cell expansion, and cell-  
570 adhesion in Arabidopsis and encodes a protein that has similarity to Sec7. *Cell* **77**, 1051-1062  
571 (1994).
- 572 25. Scarpella, E., Marcos, D., Friml, J. & Berleth, T. Control of leaf vascular patterning by polar  
573 auxin transport. *Genes Dev.* **20**, 1015-1027 (2006).
- 574 26. Fulcher, N. & Sablowski, R. Hypersensitivity to DNA damage in plant stem cell niches. *Proc.*  
575 *Natl. Acad. Sci. USA* **106**, 20984-20988 (2009).
- 576 27. Horvath, B. M. *et al.* Arabidopsis RETINOBLASTOMA-RELATED directly regulates DNA  
577 damage responses through functions beyond cell cycle control. *Embo J.* **36**, 1261-1278 (2017).
- 578 28. Carbonell, A. *et al.* New generation of artificial MicroRNA and synthetic trans-acting small  
579 interfering RNA vectors for efficient gene silencing in Arabidopsis. *Plant Physiol.* **165**, 15-29  
580 (2014).
- 581 29. Schwab, R., Ossowski, S., Riester, M., Warthmann, N. & Weigel, D. Highly specific gene  
582 silencing by artificial microRNAs in Arabidopsis. *Plant Cell* **18**, 1121-1133 (2006).
- 583 30. Brand, L. *et al.* A Versatile and reliable two-component system for tissue-specific gene  
584 induction in Arabidopsis. *Plant Physiol.* **141**, 1194-1204 (2006).
- 585 31. Moore, I., Samalova, M. & Kurup, S. Transactivated and chemically inducible gene expression  
586 in plants. *Plant J.* **45**, 651-683 (2006).
- 587 32. Jinek, M. *et al.* A programmable dual-RNA-guided DNA endonuclease in adaptive bacterial  
588 immunity. *Science* **337**, 816-821 (2012).
- 589 33. Chen, L., Wang, F., Wang, X. & Liu, Y. G. Robust one-tube Omega-PCR strategy accelerates  
590 precise sequence modification of plasmids for functional genomics. *Plant Cell Physiol.* **54**, 634-642  
591 (2013).
- 592 34. Xie, K., Minkenberg, B. & Yang, Y. Boosting CRISPR/Cas9 multiplex editing capability with  
593 the endogenous tRNA-processing system. *Proc. Natl. Acad. Sci. USA* **112**, 3570-3575 (2015).
- 594 35. Jaganathan, D., Ramasamy, K., Sellamuthu, G., Jayabalan, S. & Venkataraman, G. CRISPR for  
595 Crop Improvement: An Update Review. *Front Plant Sci.* **9**, 985 (2018).
- 596 36. Zhang, Q. *et al.* Potential high-frequency off-target mutagenesis induced by CRISPR/Cas9 in  
597 Arabidopsis and its prevention. *Plant Mol. Biol.* **96**, 445-456 (2018).
- 598 37. Shimada, T. L., Shimada, T. & Hara-Nishimura, I. A rapid and non-destructive screenable  
599 marker, FAST, for identifying transformed seeds of Arabidopsis thaliana. *Plant J.* **61**, 519-528  
600 (2010).



601 38. Kareem, A. *et al.* Protocol: A method to study the direct reprogramming of lateral root primordia  
602 to fertile shoots. *Plant Methods* **12**, 27 (2016).

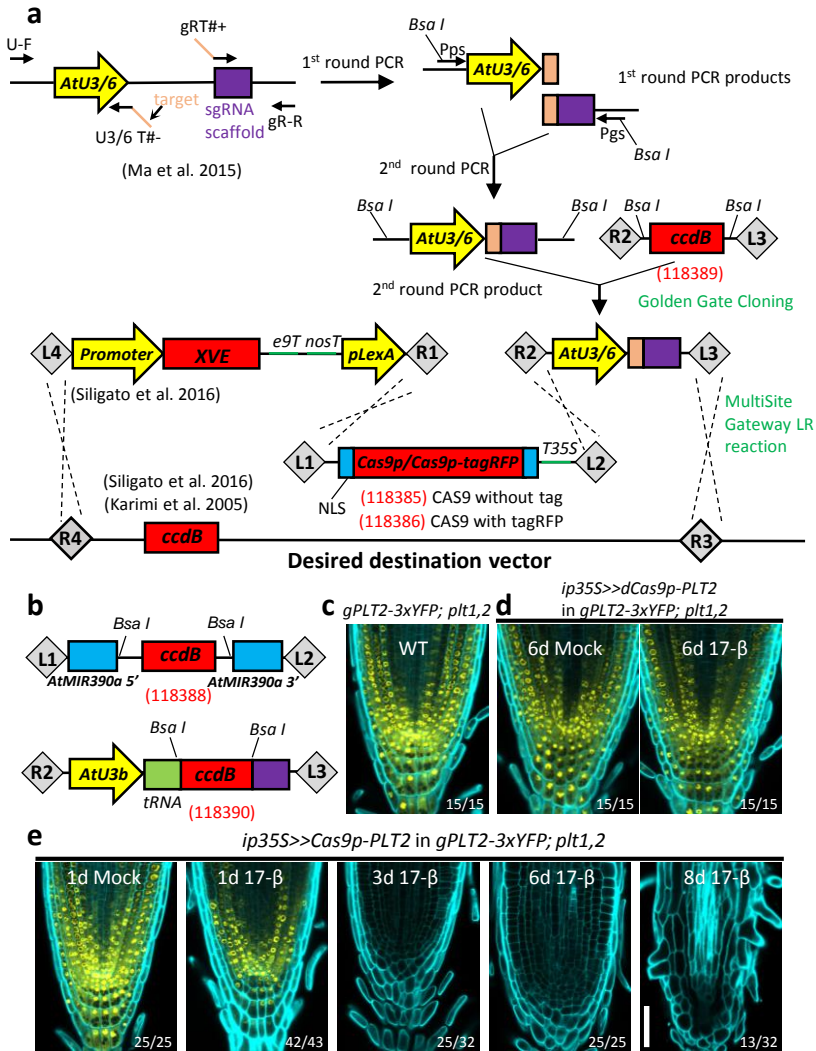
603 39. Ursache, R., Andersen, T. G., Marhavy, P. & Geldner, N. A protocol for combining fluorescent  
604 proteins with histological stains for diverse cell wall components. *Plant J.* **93**, 399-412 (2018).

605

606



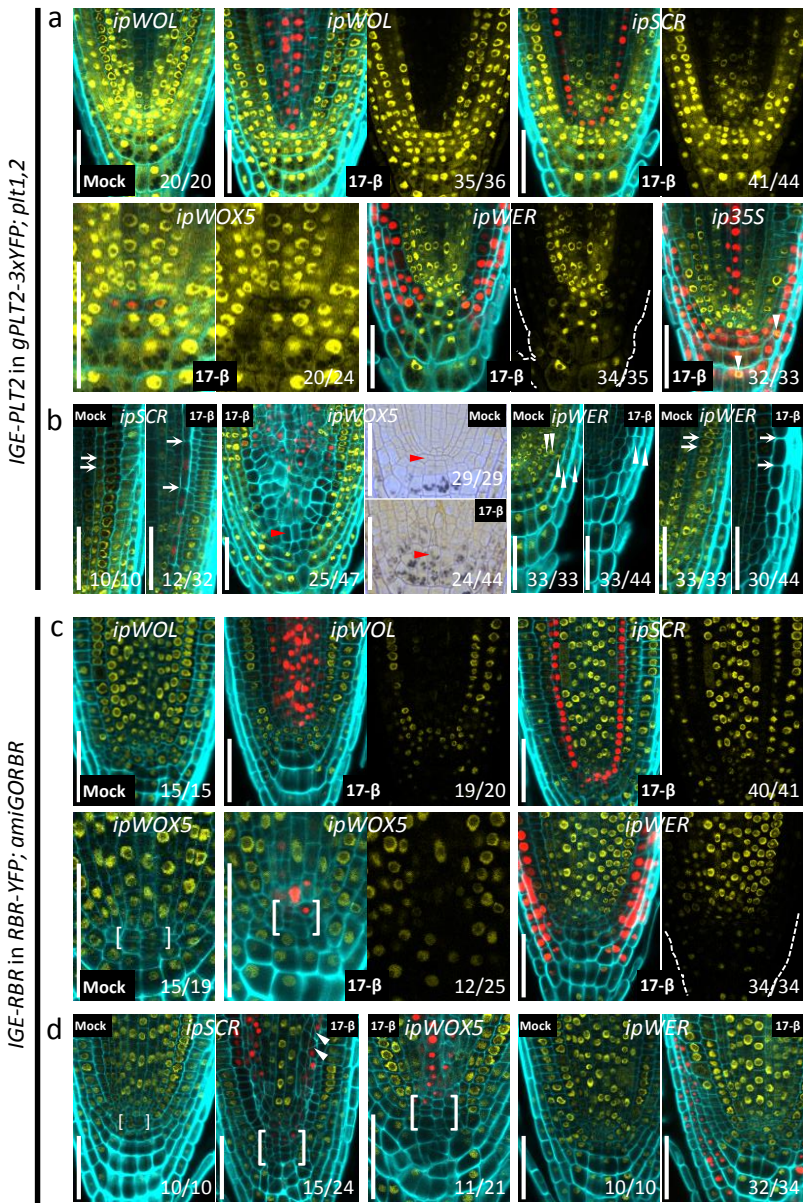
## Fig. 1



**Figure 1: Engineering the IGE system for conditional genome editing**

**a**, Cloning steps for IGE construct generation. Fusions of the sgRNA expression cassette (*pAtU3/6-sgRNA*) were constructed by two PCR steps and were subsequently cloned into the *p2R3z-Bsa I-ccdB-Bsa I* entry vector by Golden Gate cloning. The binary IGE construct was then recombined by a MultiSite Gateway LR reaction. **b**, Schematics of two other entry vectors generated in this study. Entry vector *p221z-AtMIR390a*, in which *AtMIR390a* is split by a *Bsa I*-flanking-*ccdB* cassette, was utilized for inducible gene knockdown. Entry vector *p2R3z-AtU3b-tRNA-ccdB-gRNA* was generated to exploit the endogenous tRNA processing system. Two annealed overlapping target sequences with overhangs can be directly ligated into *Bsa I*-linearized *p2R3z-AtU3b-tRNA-ccdB-gRNA*. Red numbers in brackets are the Addgene numbers of vectors created in this study. **c**, The YFP signal in the RM of 7 day-old *gPLT2-3xYFP; plt1,2*. **d**, dCas9p does not decrease PLT2-3xYFP expression. **e**, Cas9p-mediated *PLT2* editing resulted in a gradual loss of YFP and eventually full differentiation of the RM. The numbers are the frequency of the observed phenotypes in independent T1 samples. Cell walls are visualized by calcofluor. Scale bar, 50 μm.

## Fig. 2



**Figure 2: The IGE system enables efficient cell-type-specific genome editing**

**a**, A one-day induction is sufficient to induce efficient cell-type specific *PLT2* editing. In rare occasions, we observed overlapping Cas9p-tagRFP and *PLT2-3xYFP* expression (white arrowhead). **b**, *PLT2* is cell-autonomously required for QC and stem cell maintenance. QC cells (red arrowheads) as well as endodermal and epidermal cells (white arrows) showed premature differentiation or cell expansion after 3 days of induction. QC differentiation is accompanied by shift of *ipWOX5* expression towards the provascular cells. Removal of *PLT2* from the *ipWER* expression domain also resulted in fewer LRC layers (white arrowhead) and ectopically decreased the *PLT2-3xYFP* expression. Cas9p-tagRFP expression in the LRC and epidermis was frequently undetectable. **c**, A one-day induction is sufficient to induce efficient cell-type specific *RBR* editing. Without induction, the QC frequently shows cell divisions, probably due to the heterogeneity of the complementing *RBR-YFP*. **d**, *RBR* cell-autonomously prevents QC and stem cell division. The endodermis, QC and LRC exhibited overproliferation after 3 days of induction. White arrowheads indicate rotated cell division planes in the endodermis. QC regions are marked by brackets in **c** and **d**. Cell walls are highlighted by calcofluor. The numbers represent the frequency of the observed phenotypes in independent T1 samples. Scale bars, 50  $\mu$ m.