

Supplementary Methods

To generate the *p22Iz-Cas9p-t35s* entry vector, first, *Cas9p* with two flanking nuclear localized signal (*NLS*) coding sequence and a *t35* terminator were amplified from vector

pYLCRISRPCas9P35S-B¹ with chimeric primers which contained the *attB1/attB2* adaptor at the 5' end and a 3' end complementary to *NLS* and *t35s*, respectively. The resultant PCR fragment was gel-purified and then recombined with *pDONR 221* following the instructions of the Gateway BP Clonase II Enzyme mix (Invitrogen).

Site-directed mutations were introduced to two nuclease domains of *Cas9p*, RuvC1 and HNH (D10A, H840A)², respectively, to generate d*Cas9*. To achieve this, a partial *Cas9p* fragment (61-2582, starting from ATG) was amplified with primers containing the desired mutations. The purified PCR fragment was then used as a mega-primer to amplify *p22Iz-Cas9p-t35s*. The resulting PCR product was digested by methylation-specific endonuclease Dpn I to remove the parental DNA template before transformation into competent *E.coli* DH5 α cells. The presence of mutations in *p22Iz-dCas9p-t35s* was verified by Sanger sequencing.

To insert the *tagRFP* sequence between *Cas9p* and the 3' end of the *NLS* encoding sequence located in *p22Iz-Cas9p-t35s*, *tagRFP* was first amplified from the entry vector *p2R3a-tagRFP-OcsT³* with chimeric primers consisting of a 3' end of *tagRFP*-specific oligonucleotides and a 5' end of *Cas9p/NLS*-specific oligonucleotides complementary to the flanking sequence at the insertion point. The purified PCR fragment was then used as mega-primer in the subsequent Omega PCR step⁴, which used *p22Iz-Cas9p-t35s* as the template. The PCR product was treated with Dpn I before transformation into competent *E.coli* DH5 α cells. The insertion of *tagRFP* was verified by both enzyme digestion and Sanger sequencing.

To facilitate ligation of the sgRNA expression cassette (*pAtU3/6-sgRNA*) into a Gateway entry vector, the negative selection marker, a *ccdB* expression cassette flanked by two *Bsa I* sites, was amplified from *pYLCRISRPCas9P35S-B¹* with primers containing *attB2/attB3* adaptors. After a BP

reaction with *pDONR P2R-P3z*, the reaction mixture was transformed into the *ccdB*-tolerant *E.coli* strain DB3.1. Colony PCR was performed to screen for positive colonies which had been transformed with recombined plasmids but not the empty *pDONR-P2R-P3z*. The presence of the *p2R3z-Bsa I-ccdB-Bsa I* entry vector was then further confirmed by enzyme digestion and Sanger sequencing.

To generate the *p221z-AtMIR390a* entry vector (Fig. 1b), a BP reaction was performed with *pDONR 221* and *pMDC123SB-AtMIR390a-B/c*⁵ (Addgene ID: 51775). *pMDC123SB-AtMIR390a-B/c* contains *AtMIR390a* 5' end and *AtMIR390a* 3' end which were split by *Bsa I*-flanking *ccdB* expression modules. After transforming DB3.1, positive colonies were screened by colony PCR followed by enzyme digestion and sequencing. Two artificial microRNA against *PLT2* (*amiPLT2-1* and *amiPLT2-2*) were designed using <http://p-sams.carringtonlab.org/>. Annealed *amiPLT2* was ligated into *p221z-AtMIR390a* by a one-step reaction as previously described⁵.

Tandem arrayed tRNA-sgRNA units have been exploited for multiplex genome editing by using the endogenous tRNA processing machinery⁶, which precisely cuts tRNA precursors at both ends and releases free sgRNA after transcription. This strategy has been applied in a variety of plant species^{6,7}. However, to date there are few reports of its application in *Arabidopsis*. We therefore investigated its feasibility in *Arabidopsis* genome editing and meanwhile tested its compatibility with our IGE system. To facilitate target sequence ligation, we first constructed a *p2R3z-AtU3b-tRNA-ccdB-sgRNA* entry vector (Fig. 1b). *AtU3b*, tRNA-1, tRNA-2 (tRNA was amplified in two separate fragments), the *ccdB* expression cassette (flanked by *Bsa I*), and the sgRNA scaffold were amplified with the indicated primer pairs. Both ends of each fragment contained primer-introduced sequences overlapping with the desired flanking fragments. In the overlapping PCR step, *attB2-AtU3b-F* and *attB3-sgRNA-R* were used as a primer pair to assemble these five purified PCR fragments, which were mixed as templates. Cloning this fused fragment into *pDONR P2R-P3z* was conducted as described above. To clone the first target sequence of *PLT2* into *p2R3z-AtU3b-tRNA-*

ccdB-sgRNA, two annealed primers with 4-nucleotide overhangs at the 5' ends and 20-nucleotide complementary target sequences were ligated into the entry vector in a one-step reaction as described previously⁵. In the *Arabidopsis* RM, we observed a decrease of the YFP signal in the region where the inducible promoter was active in most independent lines after a 1-day induction and finally a fully differentiated RM after a 10-day induction (Extended Data Fig. 4 and Supplementary Table 1), indicating that sgRNA against *PLT2* was disassociated from tRNA processing and guiding Cas9p to cleave *PLT2*. It has recently been reported that efficient genome editing could be achieved by fusing tRNA to a mutant sgRNA scaffold but not the wild type sgRNA scaffold in *Arabidopsis*⁸. However, in our hands wild type sgRNA scaffold and tRNA fusion worked well. We reasoned that the sgRNA promoter, Cas9 variant, sgRNA scaffold, target loci, and the tissue to be edited may all affect tRNA-sgRNA-mediated editing performance in *Arabidopsis*. Therefore a future comprehensive study of these variables may improve the utility of the tRNA processing system in *Arabidopsis*.

The red seed coat vector *pFRm43GW* was generated by modifying the *pHm43GW* destination vector⁹, which was obtained from VIB (<https://gateway.psb.ugent.be/>). The *pHm43GW* vector was digested with PaeI (SphI) (ThermoFisher Scientific) to remove the hygromycin cassette. Using an In-Fusion HD Cloning (TaKaRa) kit, two fragments were cloned into the digested vector. The first fragment contained a *ccdB* cassette and recombination sites for MultiSite Gateway cloning, and it was amplified from *pHm43GW* using

GAACCCTGTGGTTGGCATGCACATACAAATGGACGAACGGATAAA as a forward primer and ATACCTACATACACTTGAAGGGTACCCGGGGATCCTCTAGAGGG as a reverse primer.

The second fragment contained the FastRed module, consisting of the *OLE1* promoter followed by *OLE1-tagRFP*, which was amplified from *pFAST-R01*¹⁰ using CTTCAAGTGTATGTAGGTATAGTAACATG as a forward primer and

CGAATTGAATTATCAGCTTGCATGCAGGGTACCATCGTTCAAACATTTGGCAAT as a reverse primer.

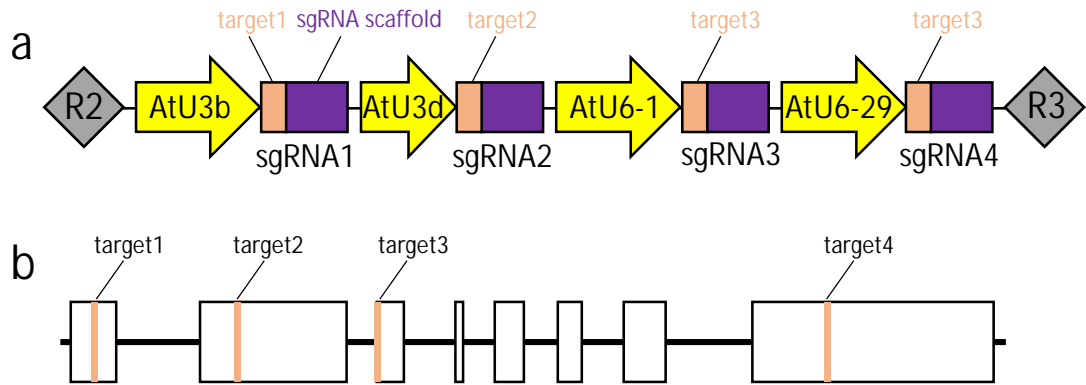
We also provide another non-destructive fluorescent screening vector, the green seed coat vector *pFG7m34GW*. It was generated by cloning the FastGreen module into the *pP7m34GW* vector⁹, which was obtained from VIB (<https://gateway.psb.ugent.be/>). The *pP7m34GW* vector was digested with SacI (ThermoFisher Scientific). Three fragments were cloned into the digested *pP7m34GW*. The first fragment contained the *OLE1* promoter followed by the *OLE1* genomic sequence and was amplified from *pFRm43GW* using CCATATGGGAGAGCTCCTTCAAGTGTATGTAGGTATAGT as a forward primer and GCCCTTGCTCACCATAGTAGTGTGCTGGCCACCACGAG as a reverse primer; the second fragment contained the *EGFP* encoding sequence and was amplified from the *pBGWFS7* vector⁹ using ATGGTGAGCAAGGGCGAGGAGCTGT as a forward primer and ATCTATGTTACTAGATCACTTGTACAGCTCGTCCATGCC as a reverse primer; the third fragment contained the *nosT* terminator sequence and was amplified from the *p1R4-ML:XVE* vector³ using TCTAGTAACATAGATGACACCGCGCG as a forward primer and TTAACGCCGAATTGAATTCGAGCTCCATCGTTCAAACAT as a reverse primer. All three fragments were combined together with the digested vector using In-Fusion HD Cloning.

Reference

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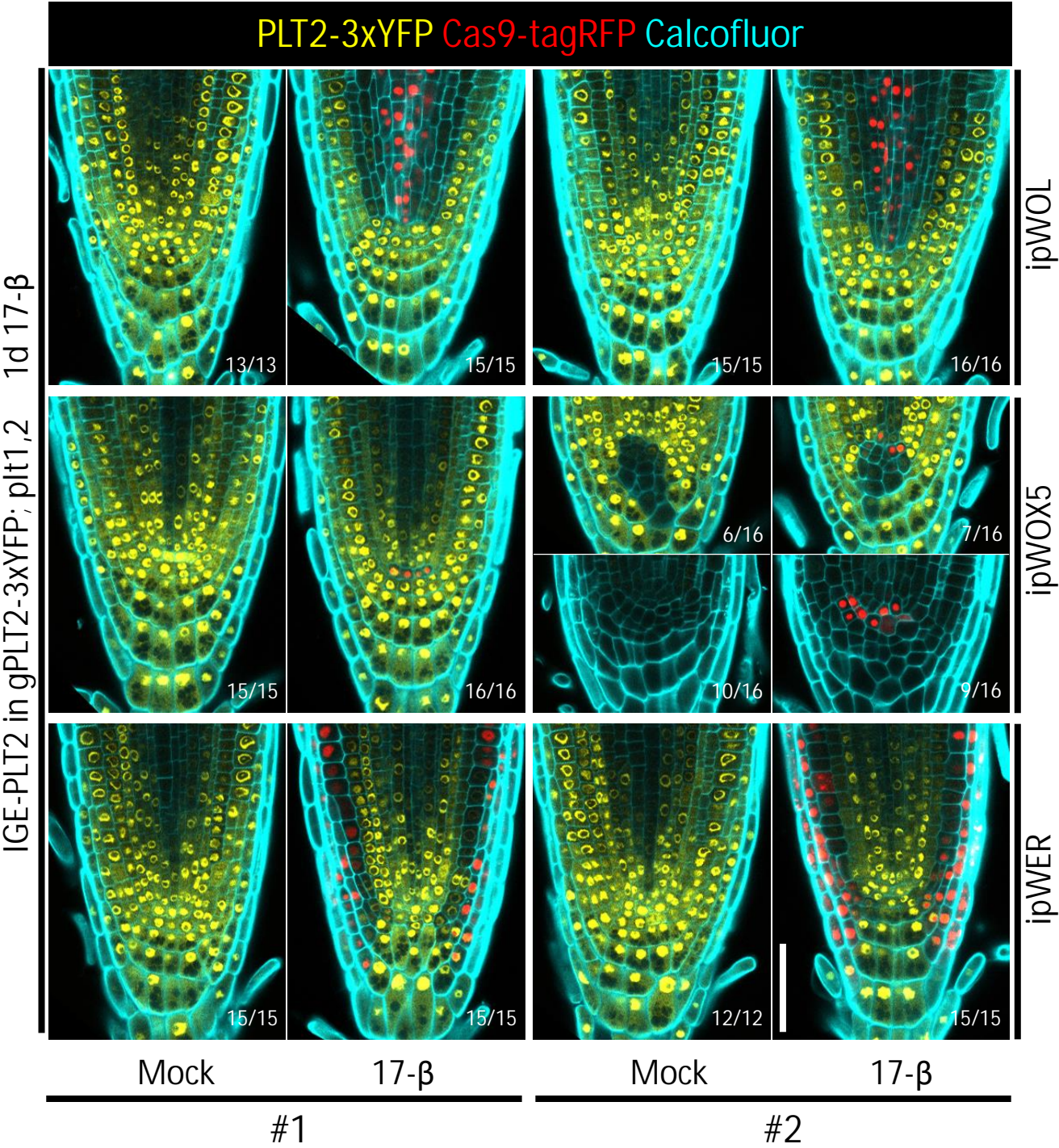
Supplementary Figure 1



Supplementary Figure 1 IGE construct targeting *PLT2*.

(a) Tandem arrayed sgRNA expression cassettes. (b) The genomic structure of *PLT2*. Boxes indicate exons. Orange bars represent targets in *PLT2*.

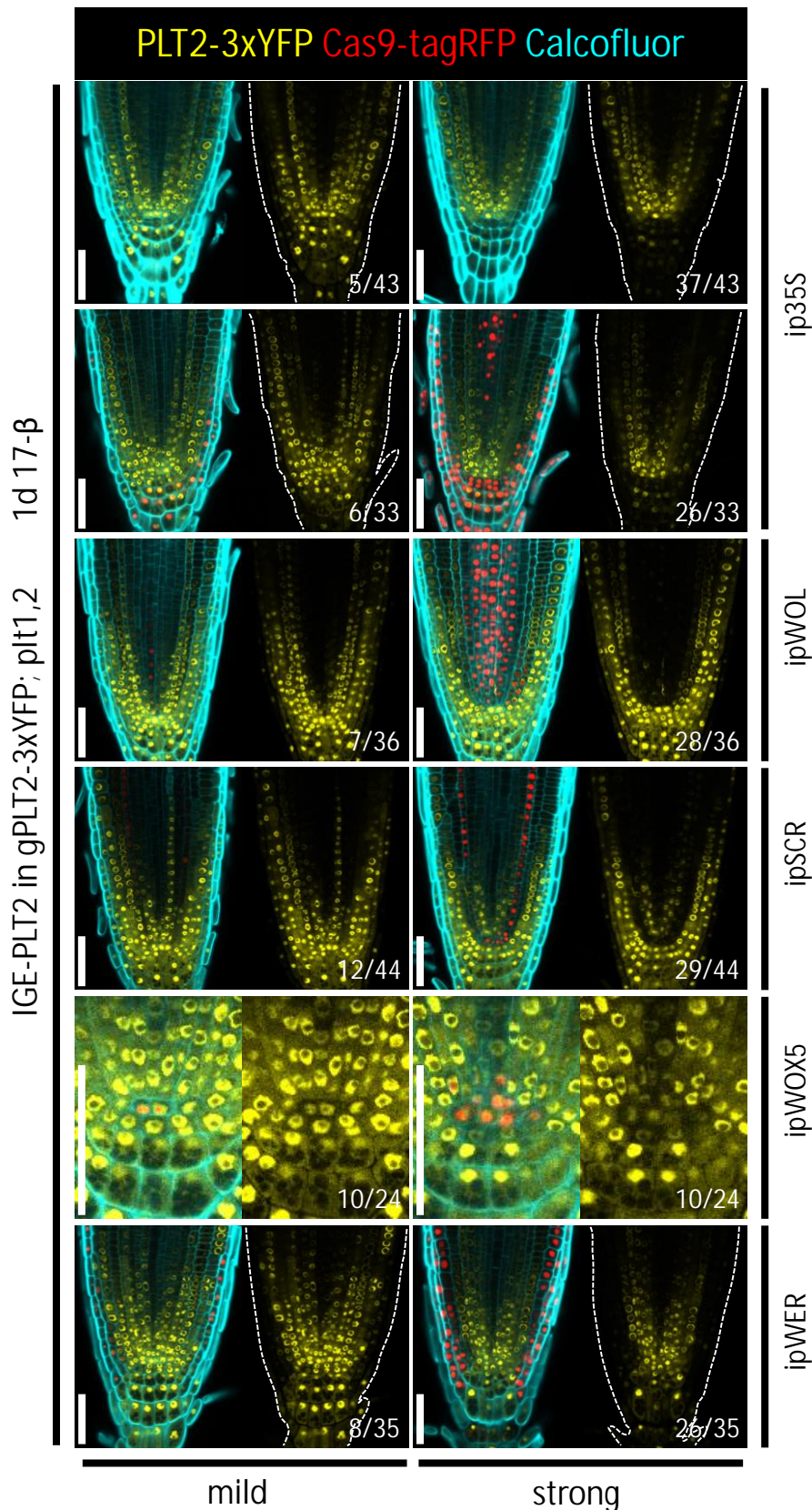
Supplementary Figure 2



Supplementary Figure 2 IGE system-driven genome editing capability is inherited.

For each construct, two independent transgenic T2 lines were randomly selected and analyzed. Representative images are shown. Note that the second *ipWOX5*>>*Cas9p-tagRFP-PLT2* line was leaky: roots displayed a similar phenotype with/without induction. Cell walls are marked by calcofluor. Numbers represent the frequency of the observed phenotype in analyzed T2 samples. All experiments were repeated three times. Scale bar, 50 μm.

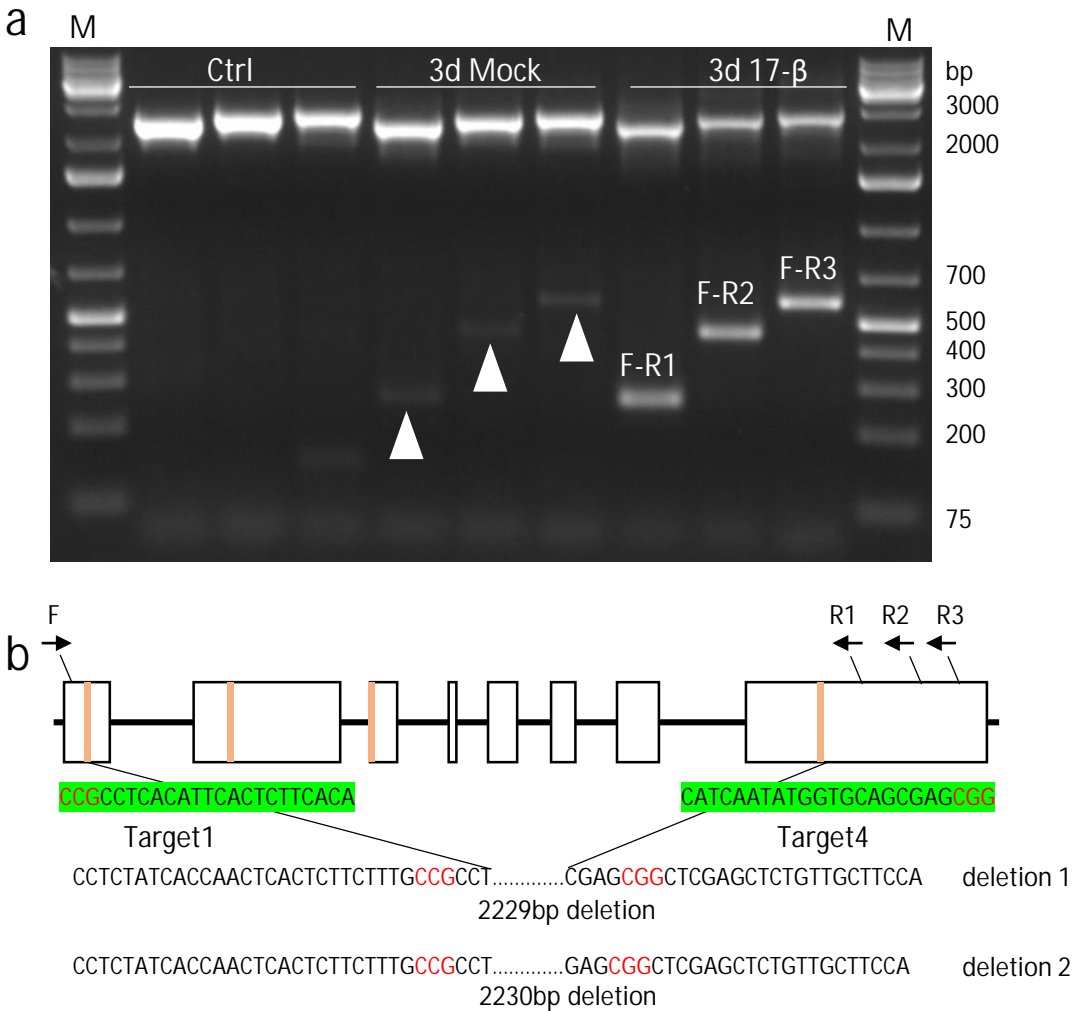
Supplementary Figure 3



Supplementary Figure 3 IGE-mediated genome editing correlates with Cas9 expression.

After one day of induction, IGE performance on *PLT2* editing under different inducible promoters was classified into two categories. In the mild category, Cas9p/Cas9p-tagRFP expression tends to be weak and narrow, resulting in narrow domains of moderately decreased YFP signal. In the strong category, Cas9p-tagRFP expression was strong and broad, with strongly and broadly reduced YFP fluorescence. In the uppermost panel, Cas9p was used without a tag. White dotted lines mark the RM outlines. Cell walls are visualized by calcofluor. Numbers indicate the frequency of similar results in the T1 samples analyzed. All experiments were repeated at least three times. Scale bars, 50 μm.

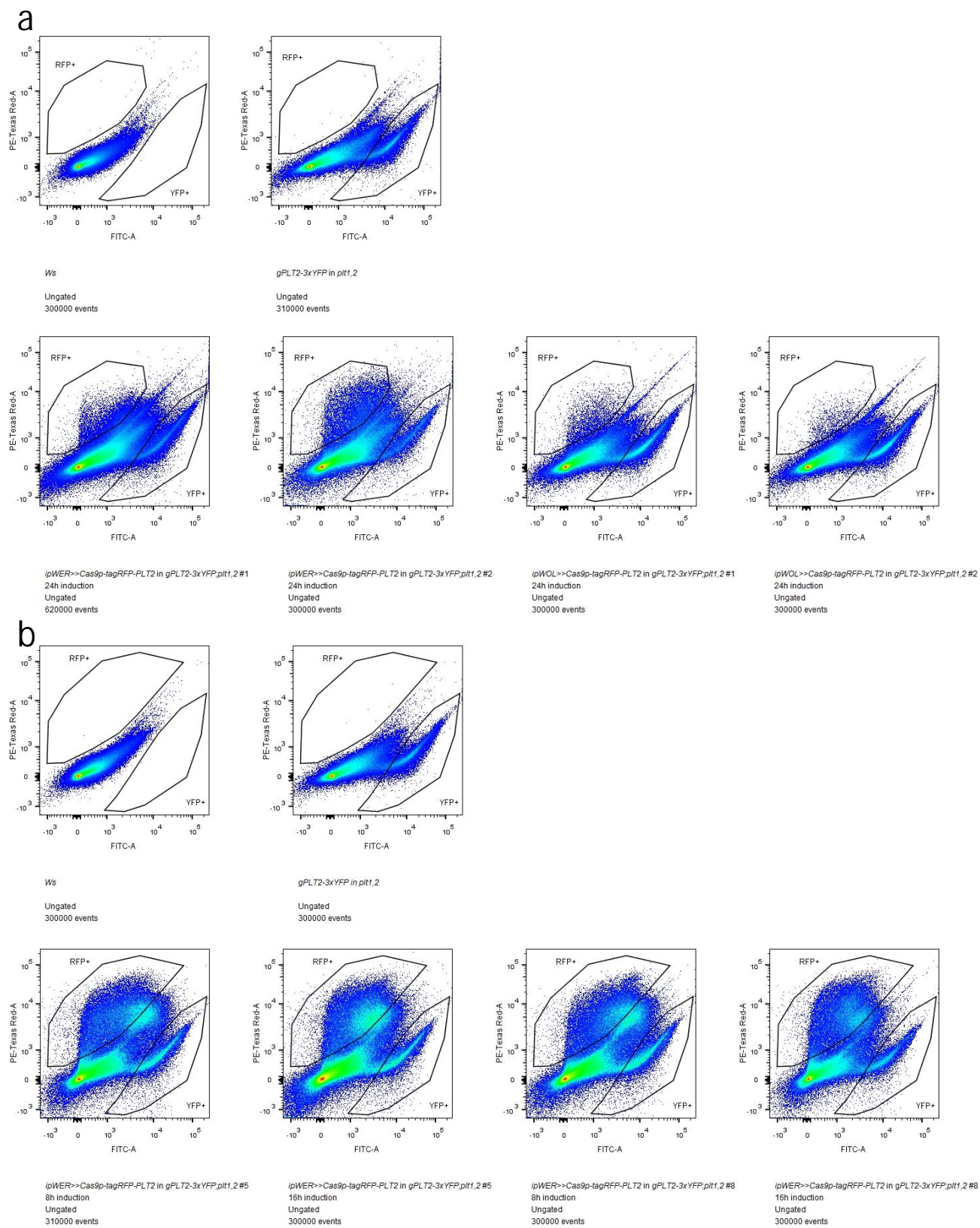
Supplementary Figure 4



Supplementary Figure 4 PCR detection of IGE-mediated genome deletion.

(a) PCR detection of *PLT2* deletion in *ip35S>>Cas9p-PLT2* in *gPLT2-3xYFP; plt1,2* T1 seedlings after 3 days of treatment (in 6 day-old plants). Pooled DNA was isolated from 2cm root segments below the hypocotyl of 10 seedlings. Three primer pairs were used. There were no detectable truncated bands in 7-day old *gPLT2 3xYFP; plt1,2* (Ctrl), while weak truncated bands were detected in mock treated seedlings (white arrowhead), probably due to weak leakiness of *ip35S* in certain roots or cells. Note that although four sgRNAs were used to target *PLT2*, only one predominant truncated band was detected with each primer pair, corresponding to deletion between target1 and target4. Experiments were repeated three times. (b) Sequencing of truncated bands from primer pair F-R3 confirmed this deletion (letters in red represent protospacer adjacent motif, PAM). To determine the deletion types, the truncated band was not directly used for sequencing but cloned into *pDONR 221*. Two deletion types were found in 4 sequenced recombinant vectors. Black arrows represent relative positions of the forward and reverse primers.

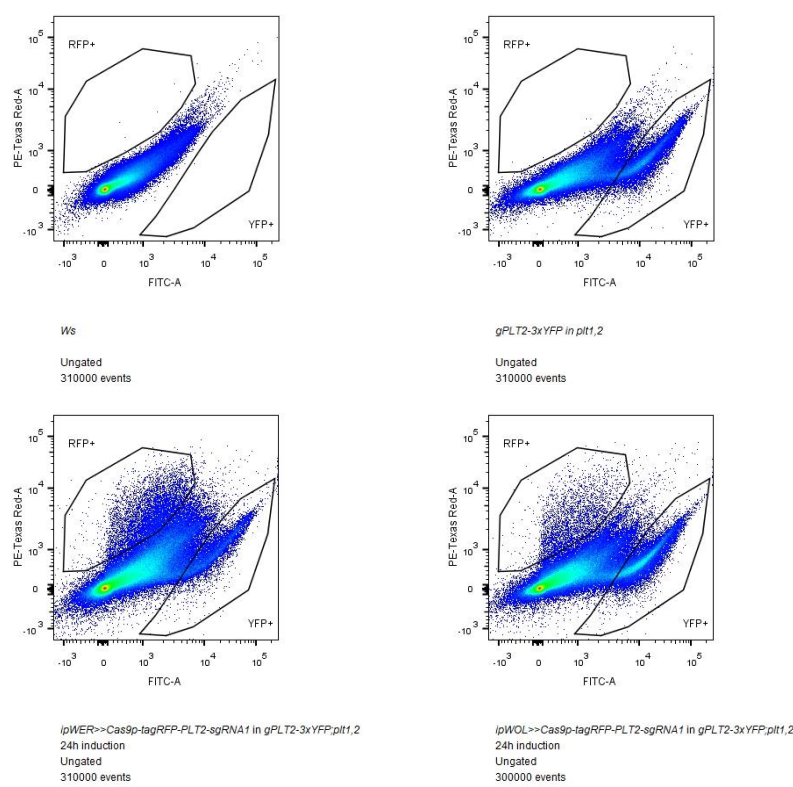
Supplementary Figure 5



Supplementary Figure 5 Fluorescence-activated cell sorting of protoplasts obtained from IGE lines.

(a) FACS of protoplasts from T2 lines of *ipWER>>Cas9p-tagRFP-PLT2 in gPLT2-3xYFP; plt1,2* and *ipWOL>>Cas9p-tagRFP-PLT2 in gPLT2-3xYFP; plt1,2* after 24h induction. (b) FACS of protoplasts from time-course 17- β induced T2 lines of *ipWER>>Cas9p-tagRFP-PLT2 in gPLT2-3xYFP; plt1,2*. Two independent transgenic lines of each construct were used for sorting. Each sample was sorted once.

Supplementary Figure 6



Supplementary Figure 6 Fluorescence-activated cell sorting of protoplasts obtained from IGE transformants containing one sgRNA.

FACS of protoplasts obtained from primary transformants (T1 generation) of *ipWER>>Cas9p-tagRFP-PLT2-sgRNA1* in *gPLT2-3xYFP; plt1,2* and *ipWOL>>Cas9p-tagRFP-PLT2-sgRNA1* in *gPLT2-3xYFP; plt1,2* after 24h induction. Sorting was performed once for each pooled T1 root material.

Supplementary Table 1 Quantification of fully differentiated root meristem (RM) after 10 days induction

1st BOX	2nd BOX	3rd BOX	Differentiated RM after 10d 17-β induction. Two repeats	
p1R4-35S:XVE	p221z-Cas9p-T35S	p2R3z-PLT2-AtU3b-sgRNA1	31/47 (66.0 %)	25/41 (61.0 %)
		p2R3z-PLT2-AtU3d-sgRNA1	17/32 (53,1 %)	20/48 (41,7 %)
		p2R3z-PLT2-AtU6-1-sgRNA1	0/29 (0.0 %)	0/43 (0.0 %)
		p2R3z-PLT2-AtU6-29-sgRNA1	15/23 (65.2 %)	22/34 (64.7 %)
		p2R3z-PLT2-AtU3b-tRNA-sgRNA1	20/34 (58.8 %)	25/31 (80.6 %)
		p2R3z-PLT2-AtU3b-sgRNA1+AtU3d-sgRNA2+AtU6-1-sgRNA3+AtU6-29-sgRNA4	17/32 (53.1 %)	25/35 (71.4 %)
	p221z-Cas9p-taqRFP-T35S	p2R3z-PLT2-AtU3b-sgRNA1	21/32 (65.6 %)	23/39 (59.0 %)
	p221z-dCas9p-T35S	p2R3z-PLT2-AtU3b-sgRNA1+AtU3d-sgRNA2+AtU6-1-sgRNA3+AtU6-29-sgRNA4	0/32 (0.0 %)	0/41 (0.0 %)
	p221z-AtMIR390-PLT2-1	p2R3z-nosT2	0/29 (0.0 %)	0/32 (0.0 %)
	p221z-AtMIR390-PLT2-2	p2R3z-nosT2	0/24 (0.0 %)	0/37 (0.0 %)

Supplementary Table 2 Primer used in this study

Primer name	sequence(5'-3')	purpose
attB1-Cas9p-T35s-F	GGGGACAAGTTTGTACAAAAAAGCAGGCTCGATGGCTCCT AAGAAGAAGCG	For cloning Cas9p with T35s terminator into 2nd BOX
attB2-Cas9p-T35s-R	GGGGACCACTTTGTACAAGAAAGCTGGGTGGTCACTGGA TTTTGGTTTAGG	
attB2-ccdB-F	GGGGACAGCTTCTTGTACAAAGTGGAACTCGAGAGACCT CTGAAGTGG	For cloning Bsa I-ccdB- Bsa I into 3 box
attB3-ccdB-R	GGGGACAACCTTTGTATAATAAAGTTGAACCGCGAGACCCA CGCTCAC	
PLT2-TG1-gRT#+	TGTGAAGAGTGAATGTGAGGTTTTAGAGCTAGAAAT	For cloning 4 sgRNA expression cassettes targeting PLT2
PLT2-TG1-AtU3bT#-	CCTCACATTCACCTTTCACATGACCAATGTTGCTCC	
PLT2-TG2-gRT#+	ATAAGGTACGAGGTTGTGATTTTTAGAGCTAGAAAT	
PLT2-TG2-AtU3dT#-	ATCACAACCTCGTACCTTATTGACCAATGGTGCTTTG	
PLT2-TG3-gRT#+	TTAGATAACTAACTACGAGAGTTTTAGAGCTAGAAAT	
PLT2-TG3-AtU6-1T#-	TCTCGTAGTTAGTTATCTAACAATCACTACTTCGTCT	
PLT2-TG4-gRT#+	CATCAATATGGTGCAGCGAGTTTTAGAGCTAGAAAT	
PLT2-TG4-AtU6-29T#-	CTCGCTGCACCATATTGATGCAATCTCTTAGTCGACT	
dCas9p-D10A-F	TACTCCATCGGCCTCgcgATCGGCACCAACAGC	dCas9 cloning
dCas9p-H840A-R	GACTGAGGAACAATcgcGTCGACGTCGTAGT	
attB1-gPLT2-F	GGGGACAAGTTTGTACAAAAAAGCAGGCTCGATGAATTCT AACAACCTGGCTC	PCR detection of PLT2 deletion from genome, and subsequent cloning into pDONR221z for sequencing
attB2-gPLT2-R1	GGGGACCACTTTGTACAAGAAAGCTGGGTGGAATCATGA TACTGAGAGAT	
attB2-gPLT2-R2	GGGGACCACTTTGTACAAGAAAGCTGGGTGGAGCTTGAC CCAATACCAAT	
attB2-gPLT2-R3	GGGGACCACTTTGTACAAGAAAGCTGGGTGGATCCTTGA GCAGACTCTCC	
amiPLT2-1-F	TGTATGATGATCCCCGATTTGCTGATGATGATCACATTG TTATCTATTTTTTCAGCAAATCGTGGGATCATCA	amiPLT2-1 cloning
amiPLT2-1-R	AATGTGATGATCCACGATTTGCTGAAAAAATAGATAACG AATGTGATCATCATCAGCAAATCGGGGGATCATCA	
amiPLT2-2-F	TGTATGATCGGTGTGATGATCCCCGATGATGATCACATTC GTTATCTATTTTTTCGGGGATCATAACACCGATCA	amiPLT2-2 cloning
amiPLT2-2-R	AATGTGATCGGTGTTATGATCCCCGAAAAAATAGATAACG AATGTGATCATCATCGGGGATCATCACACCGATCA	
PLT2-TG1-AtU3dT#-	CCTCACATTCACCTTTCACATGACCAATGGTGCTTTG	sgRNA promoter comparison
PLT2-TG1-AtU6-1T#-	CCTCACATTCACCTTTCACACAATCACTACTTCGTCT	
PLT2-TG1-AtU6-29T#-	CCTCACATTCACCTTTCACACAATCTCTTAGTCGACT	
YFP-gRT	CCCATCTGGTCGAGCTGGA	YFP targeting
AtU3b-YFP	TCCAGCTCGACCAGGATGGGTGACCAATGTTGCTCC	
RBR-TG1-gRT#+	TCAGCAAGCATGTCTAACATGTTTTAGAGCTAGAAAT	For cloning 4 sgRNA expression cassettes targeting RBR
RBR-TG1-AtU3bT#	ATGTTAGACATGCTTGCTGATGACCAATGTTGCTCC	
RBR-TG2-gRT#+	GTC AAGGCTGGATCTGTACTGTTTTAGAGCTAGAAAT	
RBR-TG2-AtU3dT#	AGTACAGATCCAGCCTTGACTGACCAATGGTGCTTTG	
RBR-TG3-gRT#+	TATCCTCAACTCATCTTCTGTTTTAGAGCTAGAAAT	
RBR-TG3-AtU6-1T#	CAGAAGATGAGTTGAGGATACAATCACTACTTCGTCT	
RBR-TG4-gRT#+	TATGACAGTCTGAGCCACTGTTTTAGAGCTAGAAAT	
RBR-TG4-AtU6-29T#	AGTGGCTCAGGACTGTCATACAATCTCTTAGTCGACT	
GNOM-TG1-gRT#+	ACTACACTTGTCAACAGAGCGTTTTAGAGCTAGAAAT	For cloning 4 sgRNA expression cassettes targeting GNOM
GNOM-TG1-AtU3bT#	GCTCTGTTGACAAAGTGATTTGACCAATGTTGCTCC	
GNOM-TG2-gRT#+	TTGATGGATGATGGACCACTGTTTTAGAGCTAGAAAT	
GNOM-TG2-AtU3dT#	ACTGGTCCATCATCCATCAATGACCAATGGTGCTTTG	
GNOM-TG3-gRT#+	GTGTA CT CATCAAGATGGACGTTTTAGAGCTAGAAAT	
GNOM-TG3-AtU6-1T#	GTCCATCTTGATGAGTACACCAATCACTACTTCGTCT	
GNOM-TG4-gRT#+	TCAGCTCATCTACAGTCAATGTTTTAGAGCTAGAAAT	
GNOM-TG4-AtU6-29T#	ATTGACTGTAGATGAGCTGACAATCTCTTAGTCGACT	

attB2-AtU3b-F	<u>GGGGACAGCTTTCTTGACAAAGT</u> <u>GGAATTTACTTTAAATT</u> <u>TTTTCTTAT</u>	Generating p2R3z- AtU3b-tRNA-ccdB-gRNA entry clone
tRNA-AtU3b-R	ACCACTAGACCACTGGTGCTTTGTTTGACCAATGTTGCTCC CTCAGTGTT	
AtU3b-tRNA-F	TAACACTGAGGGAGCAACATTGGTCAAACAAAGCACCAGT GGTCTA	
tRNA-R	CCGTGGCAGGGTACTATTCTACCACTAGACCACTGGTGCT TTGTT	
tRNA-F	AGAATAGTACCCTGCCACGGTACAGACCCGGGTTTCGATT CCGGCT	
ccdB-tRNA-R	TGAATCGGCCACTTCAGAGGTCTCTTGCAACAGCCGGGAA TCGAACCCGGG	
tRNA-ccdB-F	CCCGGGTTCGATTCCCGGCTGGTGCAAGAGACCTCTGAAG TGGCCGATTCA	
ccdB-sgRNA-R	AAC TTGCTATTTCTAGCTCTAAAACCGAGACCCACGCTCAC CCGCCGCGC	
ccdB-sgRNA-F	GCGCGGCGGGTGAGCGTGGGTCTCGGTTTTAGAGCTAGA AATAGCAAGTT	
attB3-sgRNA-R	<u>GGGGACAAC</u> <u>TTTGTATAATAAAGTT</u> <u>GAAAAAAAAAAGCAC</u> <u>CGACTCGGTGCCA</u>	
BSAI-PLT2-TG1-F	TGCA <u>TGTGAAGAGTGAATGTGAGG</u>	For cloning PLT2 target 1 into 2R3z-AtU3b- tRNA-ccdB-gRNA entry clone
BSAI-PLT2-TG1-R	AAACCCTCACATTCACCTCTTCACA	
Cas9-RFP-F	CGTATCGACCTTTCCAGCTTGGTGGTGATATGAGCGAGC TGATTAAGGA	For making p221z- Cas9p-tagRFP entry clone
NLS-RFP-R	TCCGGCCTTTTGGTGGCAGCAGGACGCTTCTTG TCCCC AGTTTGCTAG	
PLT2-TG1-F1	GCTTTGATTCCAAGAAAAGGG	TIDE analysis or amplicon sequencing
PLT2-TIDE-TG1-R1	CATGTGCAATGATGCTTTTCGA	
PLT2-TIDE-TG1-R2	GTGGATTGATCATATTCCATC	
PLT2-TIDE-TG2-F	GATGGAATATGATCAATCCAC	
PLT2-TIDE-TG2-R	CTACCGGTCCATCTATGTCT	
PLT2-TIDE-TG3-F	GTGGGTATGACAAAGAAGAG	
PLT2-TIDE-TG3-R	CTTACTGAATGTTCCAAGTAG	
PLT2-TIDE-TG4-F	GCACGGAGGAAGAAGCAGCAG	
PLT2-TIDE-TG4-R	GAGCTTGACCCAATACCAAT	Amplicon sequencing Quantification of genome deletion of transgenic PLT2
PLT2-TG1-F2	ATGAATTCTAACAACCTGGCTCG	
PLT2-TG1-R	ATGTCTTAATATTTGAACCTTCG	
PLT2-qPCR-F	TGTACAAAAAAGCAGGCTTCATG	
PLT2-qPCR-R	GTTGACCAAACCTAGATTGAAATG	

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

Supplementary Table 3 Constructs generated in this study

Expression vector name	1st BOX	2nd BOX	3rd BOX	Destination vector
35S:XVE>>Cas9p-PLT2-AtU3b-sgRNA1	p1R4-35S:XVE	p221z-Cas9p-T35S	p2R3z-PLT2-AtU3b-sgRNA1	pBm43GW
35S:XVE>>Cas9p-PLT2-AtU3d-sgRNA1	p1R4-35S:XVE	p221z-Cas9p-T35S	p2R3z-PLT2-AtU3d-sgRNA1	pBm43GW
35S:XVE>>Cas9p-PLT2-AtU6-1-sgRNA1	p1R4-35S:XVE	p221z-Cas9p-T35S	p2R3z-PLT2-AtU6-1-sgRNA1	pBm43GW
35S:XVE>>Cas9p-PLT2-AtU6-29-sgRNA1	p1R4-35S:XVE	p221z-Cas9p-T35S	p2R3z-PLT2-AtU6-29-sgRNA1	pBm43GW
35S:XVE>>Cas9p-PLT2-AtU3b-tRNA-sgRNA1	p1R4-35S:XVE	p221z-Cas9p-T35S	p2R3z-PLT2-AtU3b-tRNA-sgRNA1	pFRm43GW
35S:XVE>>Cas9p-PLT2-sgRNA1-4	p1R4-35S:XVE	p221z-Cas9p-T35S	p2R3z-PLT2-AtU3b-sgRNA1+AtU3d-sgRNA2+AtU6-1-sgRNA3+AtU6-29-sgRNA4	pBm43GW
35S:XVE>>dCas9p-PLT2-sgRNA1-4	p1R4-35S:XVE	p221z-dCas9p-T35S	p2R3z-PLT2-AtU3b-sgRNA1+AtU3d-sgRNA2+AtU6-1-sgRNA3+AtU6-29-sgRNA4	pBm43GW
35S:XVE>>Cas9p-tagRFP-PLT2-AtU3b-sgRNA1	p1R4-35S:XVE	p221z-Cas9p-tagRFP-T35S	p2R3z-PLT2-AtU3b-sgRNA1	pBm43GW
35S:XVE>>AtMIR390-PLT2-1-nosT2	p1R4-35S:XVE	p221z-AtMIR390-PLT2-1	nosT2	pFRm43GW
35S:XVE>>AtMIR390-PLT2-2-nosT2	p1R4-35S:XVE	p221z-AtMIR390-PLT2-2	nosT2	pFRm43GW
pWOX5:XVE>>AtMIR390-PLT2-1-nosT2	p1R4-pWOX5:XVE	p221z-AtMIR390-PLT2-1	nosT2	pFRm43GW
pWER:XVE>>Cas9p-tagRFP-PLT2-sgRNA1-4	p1R4-pWER:XVE	p221z-Cas9p-tagRFP-T35S	p2R3z-PLT2-AtU3b-sgRNA1+AtU3d-sgRNA2+AtU6-1-sgRNA3+AtU6-29-sgRNA4	pBm43GW
pWOX5:XVE>>Cas9p-tagRFP-PLT2-sgRNA1-4	p1R4-pWOX5:XVE	p221z-Cas9p-tagRFP-T35S	p2R3z-PLT2-AtU3b-sgRNA1+AtU3d-sgRNA2+AtU6-1-sgRNA3+AtU6-29-sgRNA4	pBm43GW
pSCR:XVE>>Cas9p-tagRFP-PLT2-sgRNA1-4	p1R4-pSCR:XVE	p221z-Cas9p-tagRFP-T35S	p2R3z-PLT2-AtU3b-sgRNA1+AtU3d-sgRNA2+AtU6-1-sgRNA3+AtU6-29-sgRNA4	pBm43GW
pWOL:XVE>>Cas9p-tagRFP-PLT2-sgRNA1-4	p1R4-pWOL:XVE	p221z-Cas9p-tagRFP-T35S	p2R3z-PLT2-AtU3b-sgRNA1+AtU3d-sgRNA2+AtU6-1-sgRNA3+AtU6-29-sgRNA4	pBm43GW
pWER:XVE>>Cas9p-taRFP-RBR-sRNA1-4	p1R4-pWER:XVE	p221z-Cas9p-tagRFP-T35S	p2R3z-RBR-AtU3b-sgRNA1+AtU3d-sgRNA2+AtU6-1-sgRNA3+AtU6-29-sgRNA4	pFRm43GW
pWOX5:XVE>>Cas9p-taRFP-RBR-sRNA1-4	p1R4-pWOX5:XVE	p221z-Cas9p-tagRFP-T35S	p2R3z-RBR-AtU3b-sgRNA1+AtU3d-sgRNA2+AtU6-1-sgRNA3+AtU6-29-sgRNA4	pFRm43GW
pSCR:XVE>>Cas9p-taRFP-RBR-sRNA1-4	p1R4-pSCR:XVE	p221z-Cas9p-tagRFP-T35S	p2R3z-RBR-AtU3b-sgRNA1+AtU3d-sgRNA2+AtU6-1-sgRNA3+AtU6-29-sgRNA4	pFRm43GW
pWOL:XVE>>Cas9p-taRFP-RBR-sRNA1-4	p1R4-pWOL:XVE	p221z-Cas9p-tagRFP-T35S	p2R3z-RBR-AtU3b-sgRNA1+AtU3d-sgRNA2+AtU6-1-sgRNA3+AtU6-29-sgRNA4	pFRm43GW
35S:XVE>>Cas9p-RBR-sgRNA1-4	p1R4-35S:XVE	p221z-Cas9p-T35S	p2R3z-RBR-AtU3b-sgRNA1+AtU3d-sgRNA2+AtU6-1-sgRNA3+AtU6-29-sgRNA4	pFRm43GW
pWER:XVE>>Cas9p-tagRFP-AtU3b-YFP-sgRNA	p1R4-pWER:XVE	p221z-Cas9p-tagRFP-T35S	2R3z-YFP-AtU3b-sgRNA	pFRm43GW
pWOX5:XVE>>Cas9p-tagRFP-AtU3b-YFP-sgRNA	p1R4-pWOX5:XVE	p221z-Cas9p-tagRFP-T35S	2R3z-YFP-AtU3b-sgRNA	pFRm43GW
pWOL:XVE>>Cas9p-tagRFP-GNOM-sgRNA1-4	p1R4-pWOL:XVE	p221z-Cas9p-tagRFP-T35S	p2R3z-GNOM-AtU3b-sgRNA1+AtU3d-sgRNA2+AtU6-1-sgRNA3+AtU6-29-sgRNA4	pFRm43GW

Expression vector name	1st BOX	2nd BOX	3rd BOX	Destination vector
pWER:XVE>>Cas9p-tagRFP-PLT2-sgRNA1	p1R4-pWER:XVE	p221z-Cas9p-tagRFP-T35S	p2R3z-PLT2-AtU3b-sgRNA1	pFRm43GW
pWOL:XVE>>Cas9p-tagRFP-PLT2-sgRNA1	p1R4-pWOL:XVE	p221z-Cas9p-tagRFP-T35S	p2R3z-PLT2-AtU3b-sgRNA1	pFRm43GW