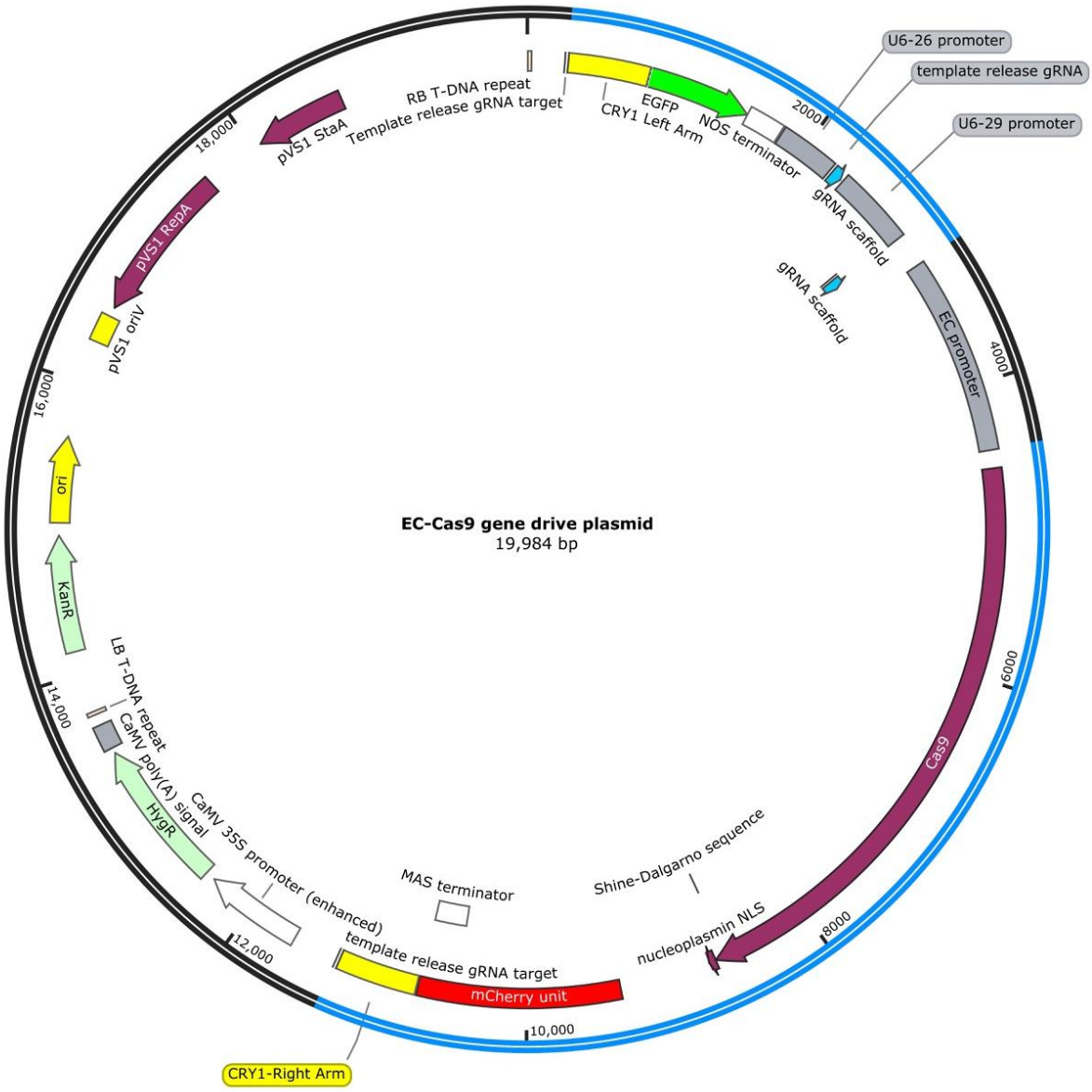
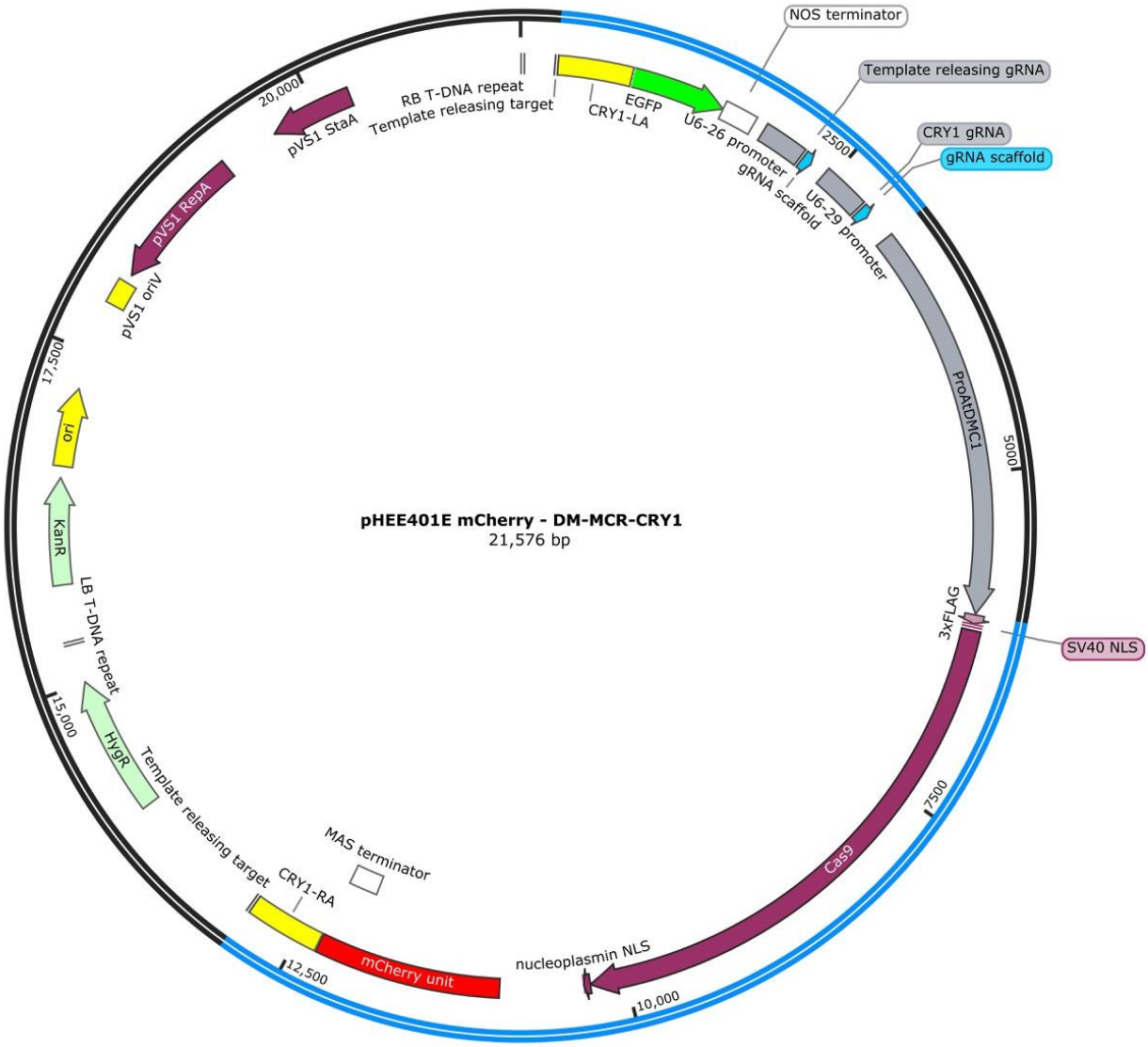
Selective inheritance of target genes from only one parent of sexually reproduced F1 progeny in *Arabidopsis*

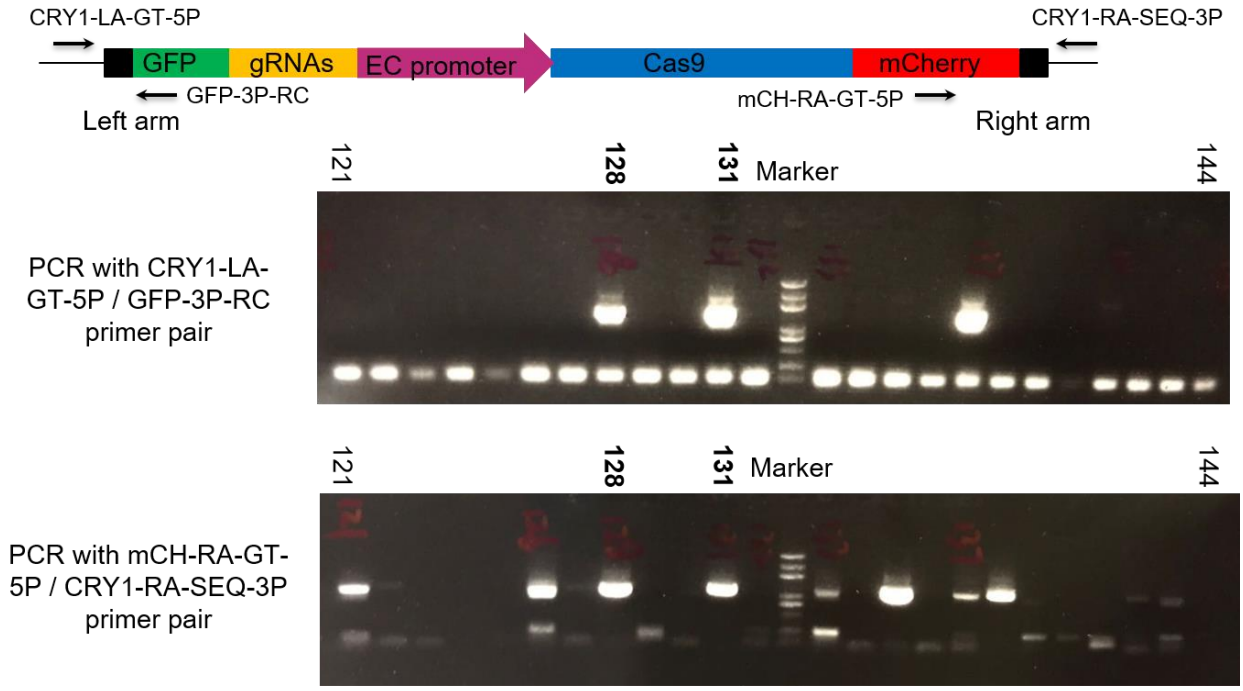
Zhang *et al.*



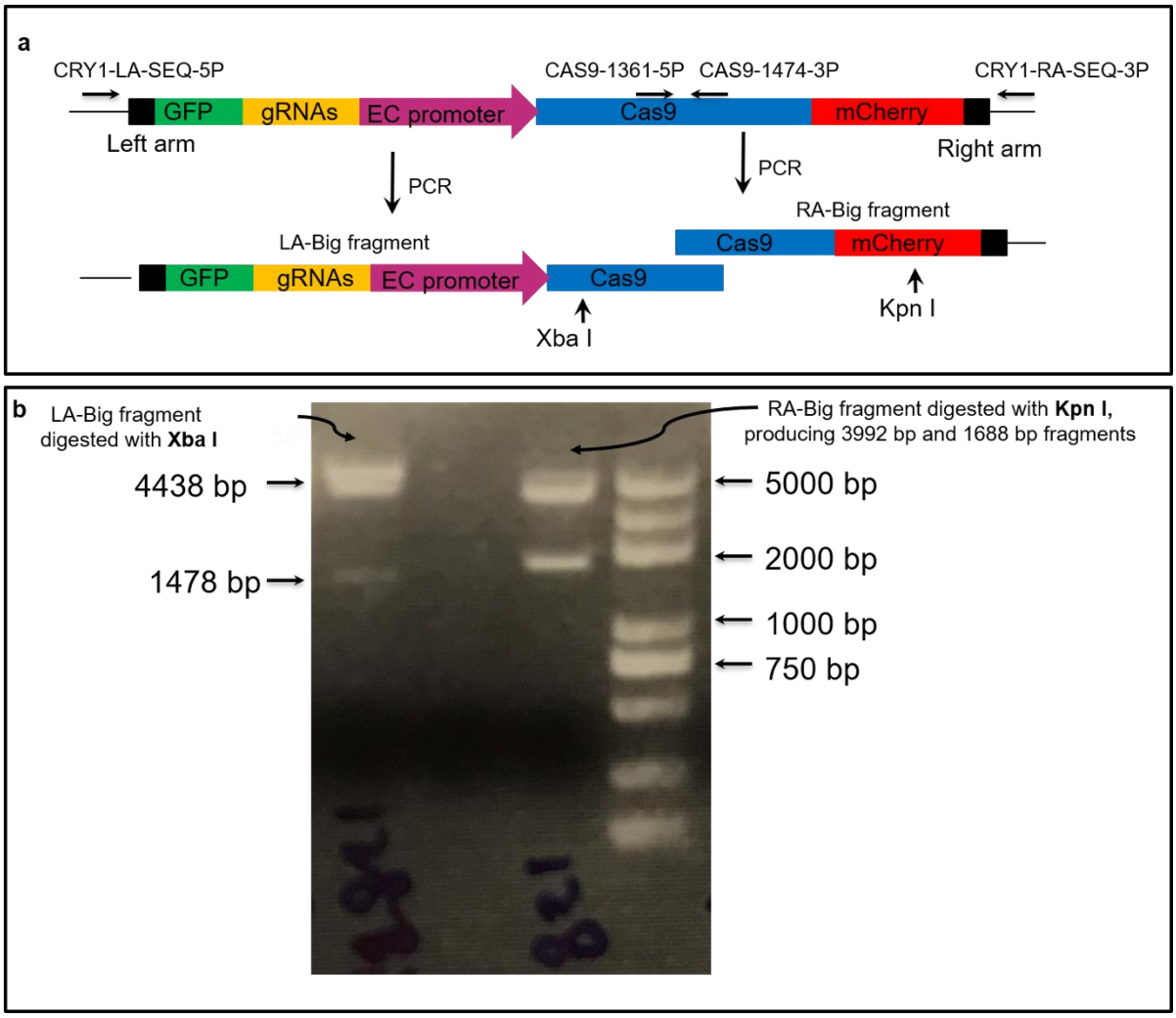
Supplementary Fig. 1. The map of the EC-Cas9 gene drive plasmid. The plasmid was designed to insert the gene drive element into the CRY1 gene in *Arabidopsis*. The gene drive element flanked with two homology arms is released by CRISPR/Cas9. The major components including the Cas9 unit, gRNA units, selection markers, and promoters are shown.



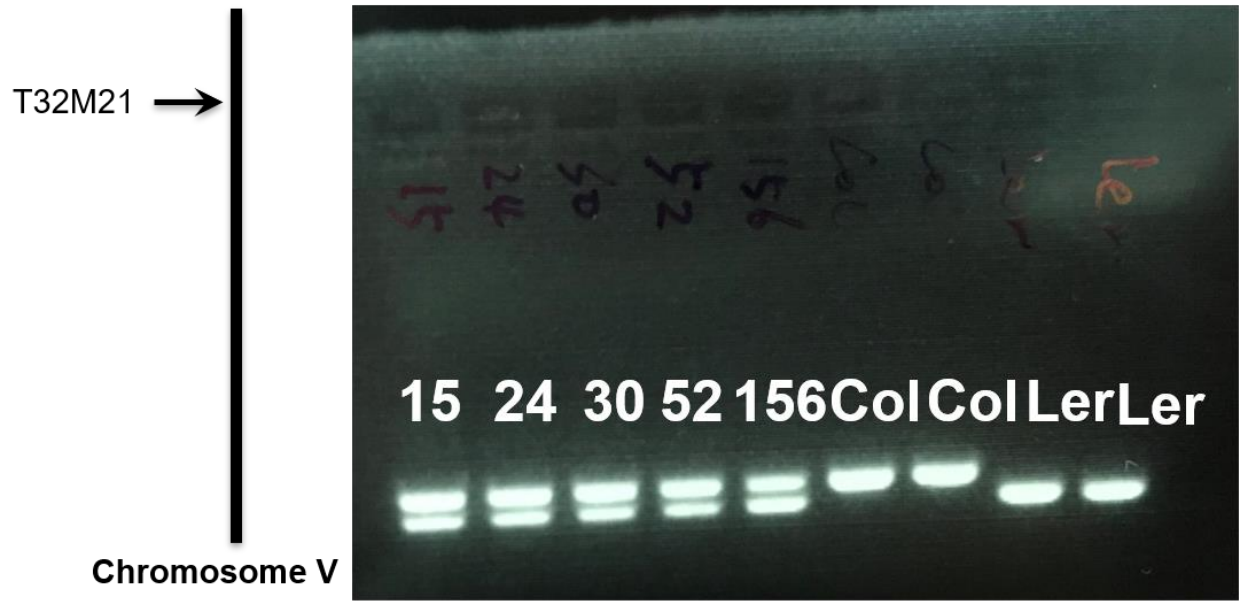
Supplementary Fig. 2. The map of the DMC-Cas9 gene drive plasmid. The plasmid was designed to insert the gene drive element into the CRY1 gene in *Arabidopsis*. The gene drive element flanked with two homology arms is released by CRISPR/Cas9. The major components including the Cas9 unit, gRNA units, selection markers, and promoters are shown.



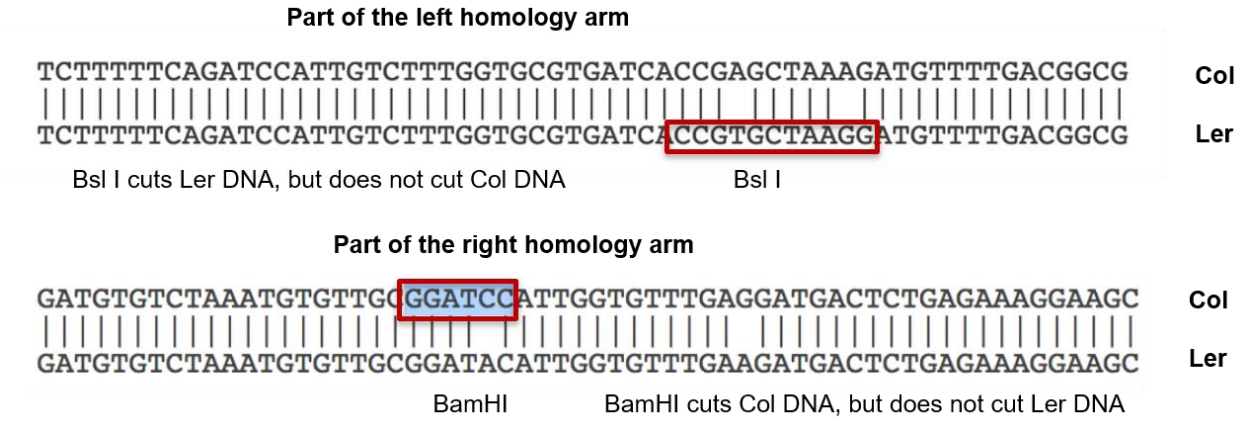
Supplementary Fig. 3. Identification of the intended insertions in the *CRY1* gene in T1 plants generated by transforming the *EC-Cas9* gene drive construct into the *DMC1-Cas9* *Arabidopsis* plants. Two sets of primers were used to amplify the left-side and right-side junctions. The *CRY1* primers were located outside of the homology arms. Only plants with the proper insertions will yield a PCR product with the right size. Some T1 plants only had HDR at one side such as #121 and some underwent HDR at both sides (#128 and #131). The positive plants were genotyped again to confirm the HDR events. Source data are provided as a Source Data file



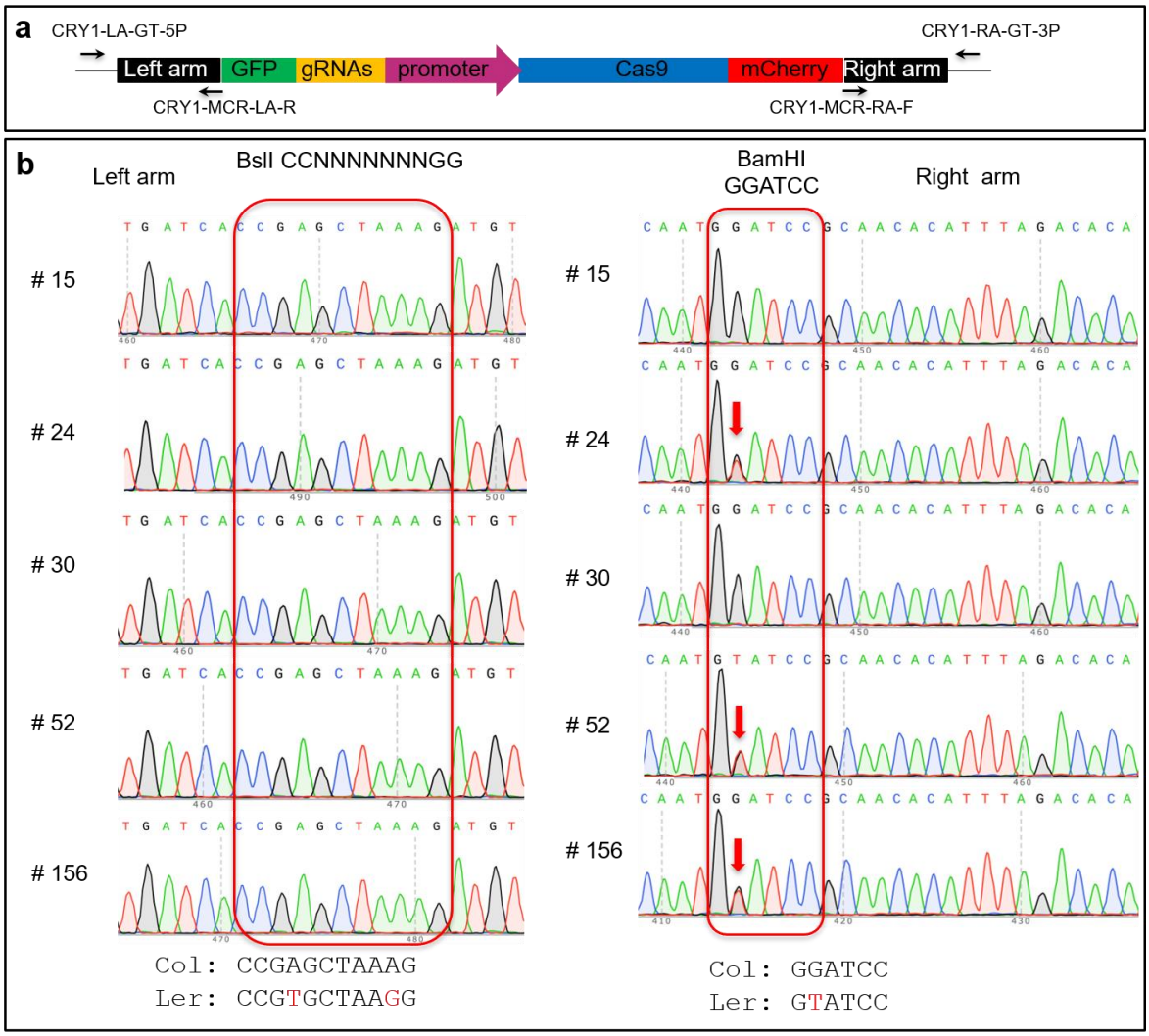
Supplementary Fig. 4. Analysis of the insertion in the *CRY1* gene in the *EC-Cas9/DMC1* #128 line. a) Two PCR reactions were used to amplify two overlapping fragments. The CRY1-LA-SEQ-5P/CAS9-1474-3P primer pair amplifies out a fragment of about 6 kb, which can be digested by XbaI, yielding two fragments with sizes of 4438 bp and 1478 bp. **b**) The PCR reaction using the CRY1-RA-SEQ-3P/CAS9-1361-5P primer pair produces a fragment of 5680 bp. KpnI digestion of the fragment produces two fragments of about 1.7 kb and 4 kb. Sequencing results of the fragments indicated that the entire gene drive element was inserted into *CRY1* in *EC-Cas9/DMC1* #128. Source data underlying Supplementary Fig. 4b are provided as a Source Data file.



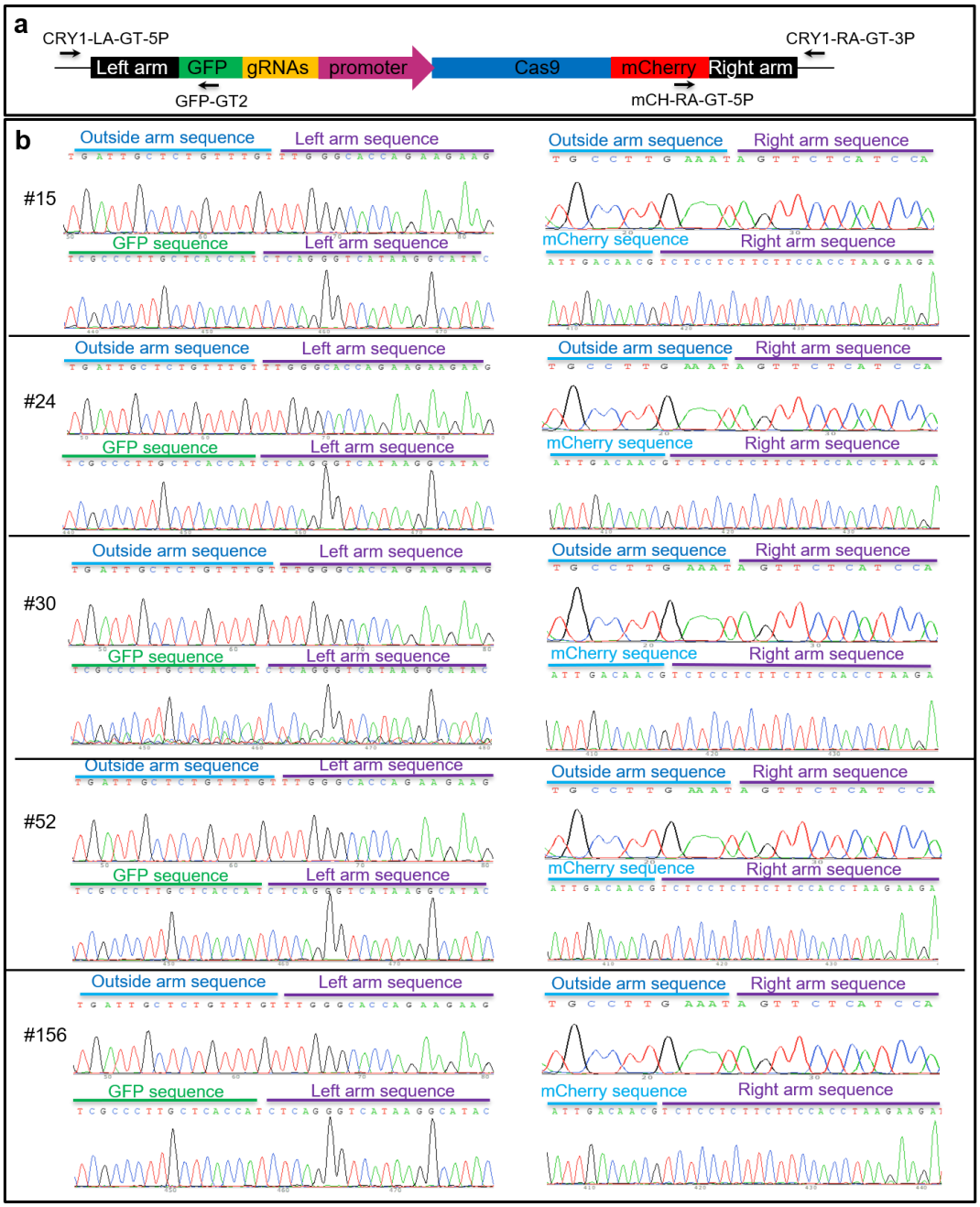
Supplementary Fig. 5. Analyses of the homozygous F1 plants from the cross between line *EC-Cas9/DMC1* #128 and wild type Ler. The Simple Sequence Length Polymorphism marker T32M21 is located on chromosome V. All five F1 homozygous plants from the cross of the *DMC1-Cas9/DD45* #128 line and Ler contain DNA from both parents, indicating that they are true F1 plants. Col refers to Columbia ecotype, and Ler is the Landsberg ecotype. The genotyping experiments were repeated three times. Source data are provided as a Source Data file



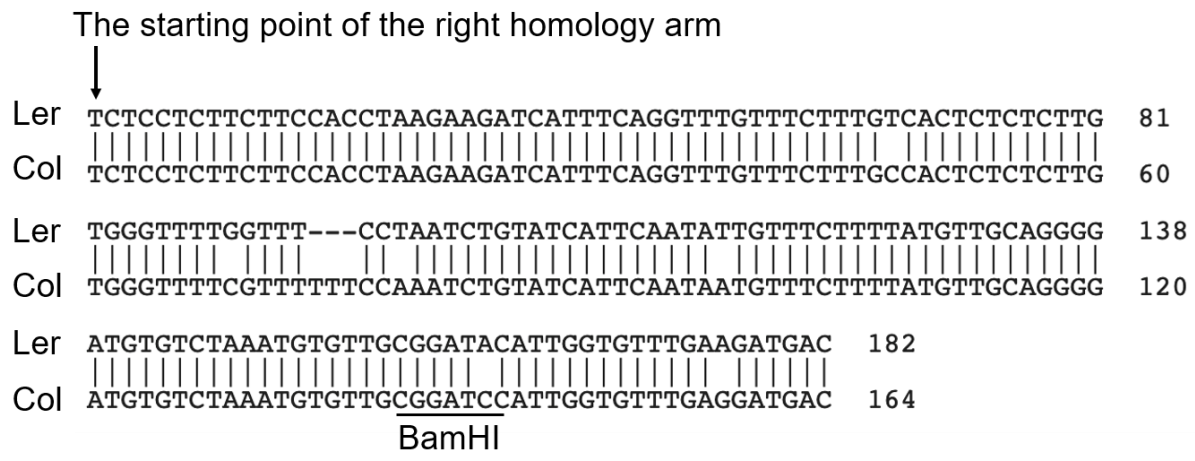
Supplementary Fig. 6. Two polymorphisms in the homology arms are used to generate two Cleaved Amplified Polymorphic Sequences (CAPS) markers. The LA marker uses BslI digestion, which cuts Ler DNA. The RA marker uses the BamHI enzyme to cut Col DNA, but not Ler DNA. The sequence differences between Ler and Col are shown.



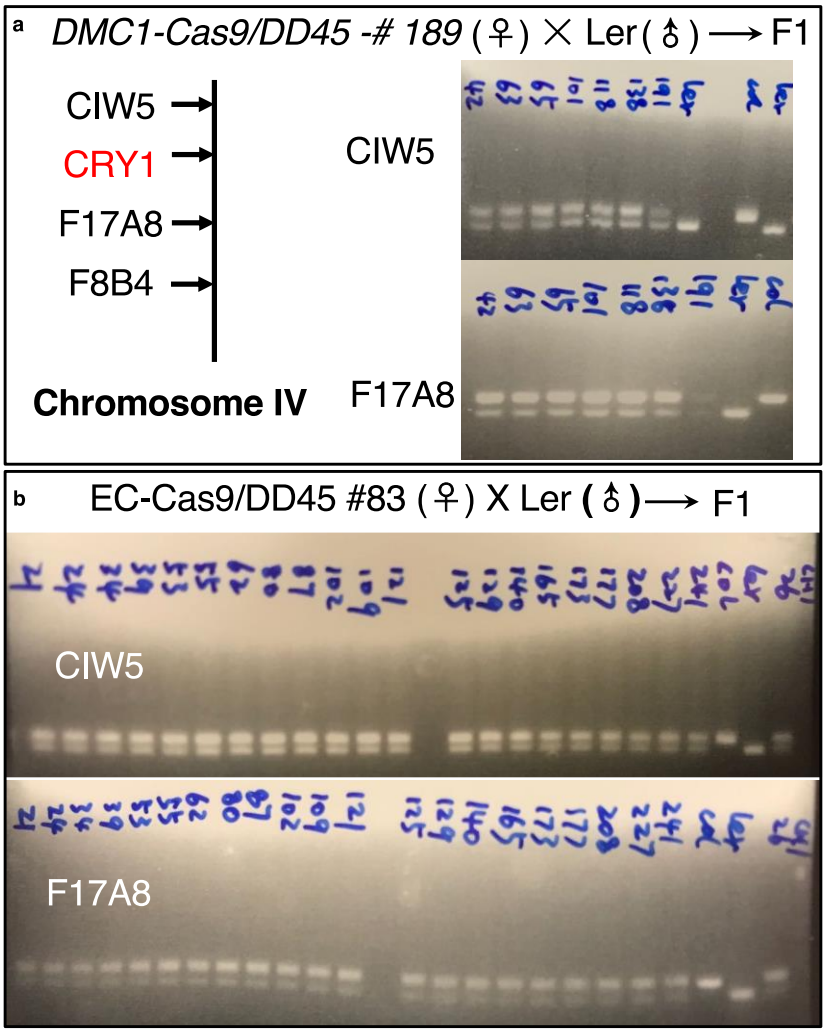
Supplementary Fig. 7. Confirmation of the CAPS markers by DNA sequencing . a) A schematic illustration of the junctions of the gene drive/genomic region and the primers used for amplification of the homology arms. **b**) Sanger sequencing results of the regions in the left homology arm and right arm with polymorphism between Col/Ler of the five F1 plants resulting from a cross between *EC-Cas9/DMC1* #128 and Ler. All F1 plants only had Col DNA in the left arm. Three of the F1 plants contained both Col and Ler DNA, as indicated by the double-peak (arrow) whereas two of the F1 plants only had Col DNA in the right arm. The left arm was amplified using CRY1-LA-GT-5P and CRY1-MCR-LA-R and the right arm was amplified with the primers CRY1-MCR-RA-F and CRY1-RA-GT-3P.



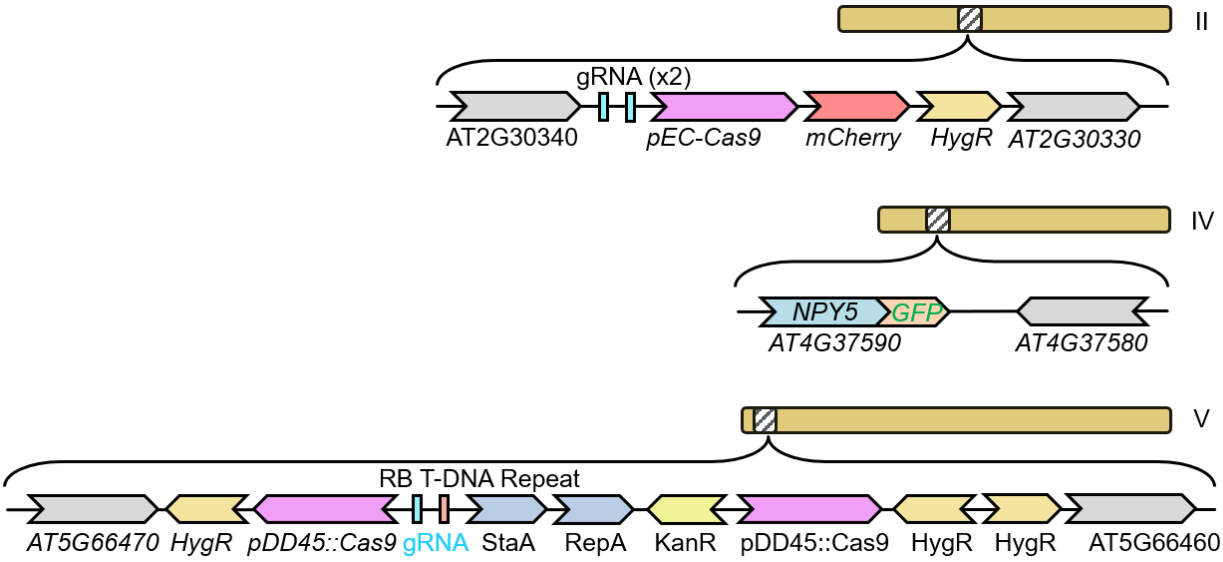
Supplementary Fig. 8. Sanger sequencing results of the junctions of F1 homozygous plants. **a**) Schematic drawing of the gene drive element inserted into the *CRY1* locus. "Left" sequences were amplified with the primers CRY1-LA-GT-5P and GFP-GT2. "Right" sequences were amplified with the primers mCH-RA-GT-5P and CRY1-RA-GT-3P. The borders of each element in the construct are indicated. **b**) Sanger sequencing results at the junctions of the five F1 homozygous plants (lines #15, #24, #30, #52, #156).



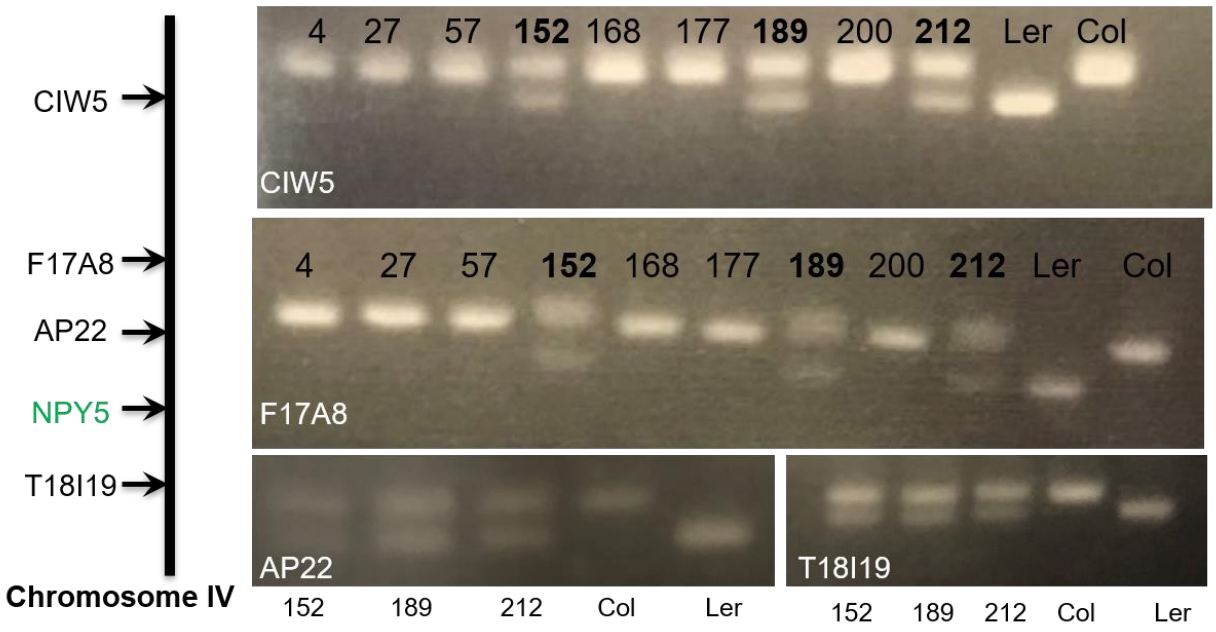
Supplementary Fig. 9. Polymorphisms between Ler and Col in the right arm. Sanger sequencing of the Ler wild type line revealed extensive polymorphisms between Ler and Col in the right homology arm. The starting point of the right homology arm is shown. The BamHI CAPS marker used in this work is also indicated.



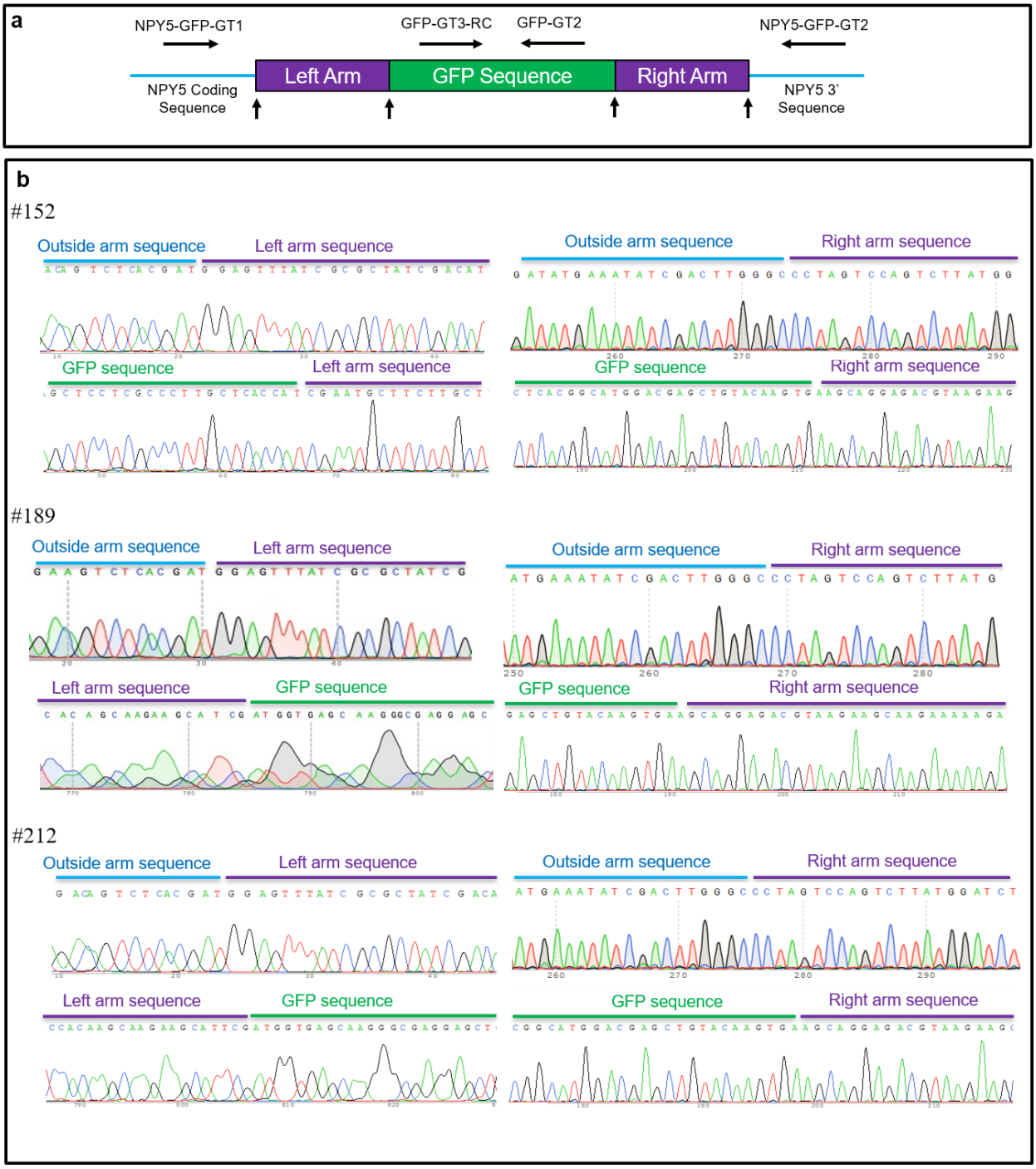
Supplementary Fig. 10. Using DNA markers to determine the origins of DNA in the F1 plants that harbored homozygous insertions at the *CRY1* locus. a) Both the CIW5 and F17A8 markers are located on chromosome 4 where the target gene *CRY1* is located. The F1 plants from the cross between *DMC1-Cas9/DD45 # 189* and Ler had DNA from both parents, indicating that they were true F1 plants and that the homozygosity at the *CRY1* locus was the result of gene drive function. **b**) Similar experiments were performed to confirm that all homozygous F1 plants from the cross between Ler and *EC-Cas9/DD45 #83* were indeed F1 plants. The genotyping experiments were repeated three times. Source data are provided as a Source Data file.



Supplementary Fig. 11. Transgene insertions in the *NPY5-GFP* line. Three transgene insertions in the *NPY5-GFP* line were uncovered by whole genome sequencing. The *GFP* gene was inserted in frame with the *NPY5* gene through HDR. No other foreign DNA was inserted into the *NPY5* locus. The second set of transgenes was located at the end of chromosome V. This insertion was complex and was generated by transforming the *DD45-Cas9* construct. Three copies of the hygromycin resistance gene were inserted in addition to two copies of *DD45-Cas9*, which probably resulted in elevated expression of *Cas9*. The third transgene cluster is located on chromosome II. This set of transgenes originated from the HDR construct for tagging *NPY5* with *GFP*. The *GFP* gene along with the homology arms in the HDR construct were deleted at the chromosome II locus. The transgenes expressing *Cas9* under the control of the egg cell specific promoter and the gRNA that targets the wild type *NPY5* gene are still intact on chromosome II. The *NPY5-GFP* line can be used to test the feasibility of a non-autonomous trans-acting gene drive in *Arabidopsis*.



Supplementary Fig. 12. DNA analysis of the putative F1 plants generated from a cross between the *NPY5-GFP* line and Ler. The *NPY5* gene is located on chromosome IV. Four markers were used to determine whether the plants were F1 plants or progeny from self-pollination of the *NPY5-GFP* line. Among the 9 homozygous *NPY-GFP* plants tested, only #152, 189, and 212 were F1 plants. The other plants were progeny from self-pollination. The genotype of the identified true F1 plants was further genotyped again to confirm the results. Source data are provided as a Source Data file.



Supplementary Fig. 13. Sanger sequencing results of the junctions of *NPY5-GFP* F1 trans-acting GD plants. a) Schematic drawing of the *NPY5-GFP* donor fragment inserted into the genome. The four junctions are indicated with small arrows. "Left" sequences were amplified with the primers NPY5-GFP-GT1 and GFP-GT2. "Right" sequences were amplified with the primers NPY5-GFP-GT2 and GFP-GT3-RC. **b**) Sanger sequencing results at the junction points of three F1 homozygous plants (lines #152, #189, #212). The plants were generated by crossing a single *NPY5-GFP* line with Ler.

Supplementary Table 1. Primers used in this study.

Experiment	Primer name	Primer sequence (5'-3')
Vector construction	MCR-CRY1-CRP1	ctagagtcgaagtagtgattg TGGAAGAAGAGGAGACTCA gttttagagctagaaatagc
	MCR-CRY1-CRP2	tgctatttctagctctaaaacGCTCCAACGTCTTCCGCAGCAATCTCTTAGTCGACTCTA
	CRY1-MCR-LA-F	aacgacggccagtgccaGCTGCGGAAGACGTTGGAGCGGGTTGGGCACCAGAAGAAGAAG
	CRY1-MCR-LA-R	TCCTCGCCCTTGCTCACCATCTCAGGGTCATAAGGCATAC
	GFP-F	ATGGTGAGCAAGGGCGAGGA
	GFP-R	TCACTTGTACAGCTCGTCCA
	CRY1-MCR -NOS-F	TGGACGAGCTGTACAAGTGA GATCGTTCAAACATTTGGCA
	CRY1-MCR -NOS-R	ttgtgcggaaggcaagtcga GATCTAGTAACATAGATGAC
	CRY1-MCR-RA-F	Gtattaattgattgacaacg TCTCCTCTTCTTCCACCTAA
	CRY1-MCR-RA-R	gctatgacatgattacgCCCGCTCCAACGTCTTCCGCAGCAGTTCTCATCCACAGCCCAA
	AtDMC1P-MCR- CRY1-F	AAACCAATACCATGGTTATAtggtagagtcatgttactta
	AtDMC1P-MCR-CRY1-R	tcgtggtccttgtaatccattttctcgctctaagagtctc
Genotyping and sequencing	pHEE-CAS9-1361-5P	ACATTCGATAACGGCAGCATCCCACACCAGATTCA
	CRY1-RA-SEQ-3P	TACTTCATCCCCCATCTCCATGGTAATTGAAGCAC
	CRY1-LA-SEQ-5P	CTGGTTGTGGTTCTGGTGGTTGTAGTATTGTATGG
	pHEE-CAS9-1474-3P	CGACGTAGTACGGGATCCTGAAAGTCAGAATCTTCT
	CRY1-LA-GT-5P	GTATCTGGTTGTGGTTCTGGTGG
	GFP-3P-RC	TCACTTGTACAGCTCGTCCATGC
	mCH-RA-GT-5P	CAAGACCACCTACAAGGCCAAGA
	CRY1-RA-GT-3P	CCCAGAAATACTTCATCCCCCAT
	pHEE-Pme1-LA-F	caagctcaagctgctctagc
	pHEE-Ecor1-RA-R	GAGTAGACGAGAGTGTCGTG
	NPY5-GFP-MHGT1	TTCTCGAAGCTTTGGTCTGGT
	NPY5-GFP-MHGT2	ATAGGATGAACATTTTAGCAAACCT
	NPY5-GFP-GT1	CAGAAGTTCCTCTCACTTGCGG
	NPY5-GFP-GT2	CACAATGACGGTGGCAAGTC
	GFP-GT2	ATCTTGAAGTTCACCTTGATGCCG
	GFP-GT3-RC	GACGTAAACGGCCACAAGTTCAGC
Genetic markers	T32M21-F	AAACGTTAAATTTTAGTCGGTGAGT
	T32M21-R	TCTCCGTTGCTTAGAACATTTG
	CIW5-F	GGTTAAAAATTAGGGTTACGA
	CIW5-R	AGATTTACGTGGAAGCAAT
	F17A8-F	CAGTTTGTTAAGTCTCCTCGAGTT
	F17A8-R	TTGTACATCAAATAGAGCCGCAT
	F8B4-F	CTGTAATATCAATTTTATAGCACG
	F8B4-R	AGCACTCAATAGAGACATTCA
	AP22A-F	GACTGTCCACAAGACTCCACAAGACTCCTCACTA
	AP22A-R	TATGGCTGAATATTGTTATGGGTT
	T18I19-F	AGTTCTGATCAAATTAGAAATCT
	T18I19-R	TATCAATCAATCGAATTAATAACA
	F22I13-F	TATATCTCAGGTACTTAATTTGCA
	F22I13-R	TTACCTGCATATATGTTAATTGAT