

Supporting information

Fig. S1

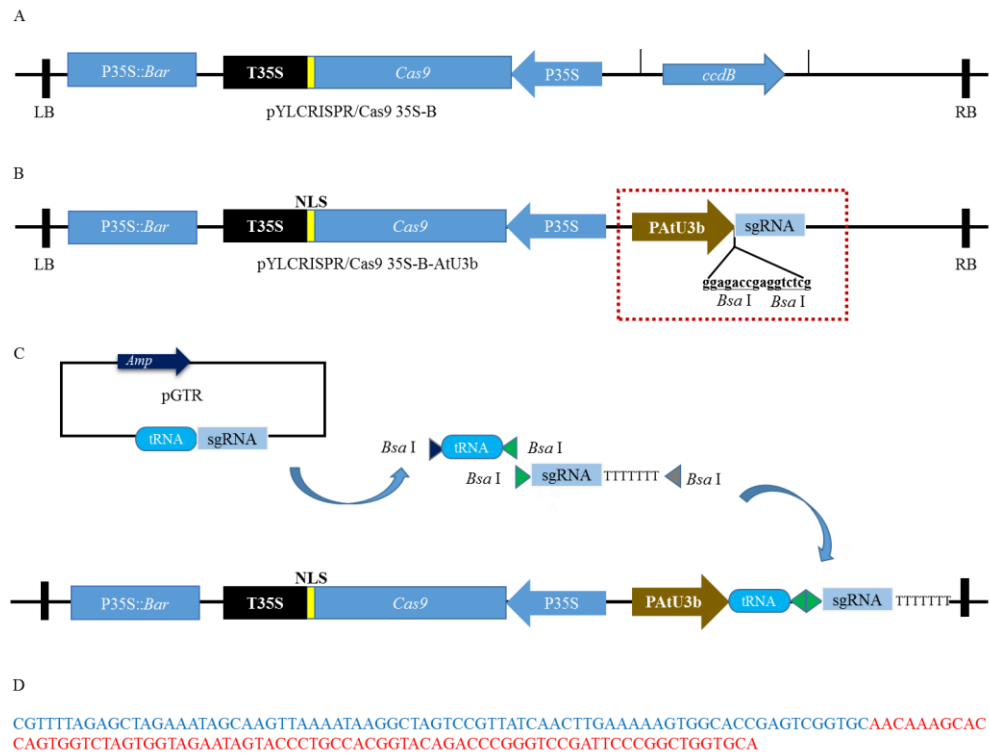


Figure S1 Schematic description of the modified pYLCRISPR/Cas9 35S-B system.

(A) Schematic illustration of pYLCRISPR/Cas9 35S-B. The red box indicates the region that was modified in the present study. NLS, nuclear localization sequence. (B) Schematic illustration of the modified CRISPR/Cas9 construct used in the present study. The red box indicates the region that was modified in the present study. NLS, nuclear localization sequence. (C) Schematic illustration of the pGTR plasmid containing the tRNA and sgRNA scaffolds, and the process for constructing the expression vector comprising both the tRNA-gRNA expression cassette and Cas9. NLS, nuclear localization sequence.

Fig S2

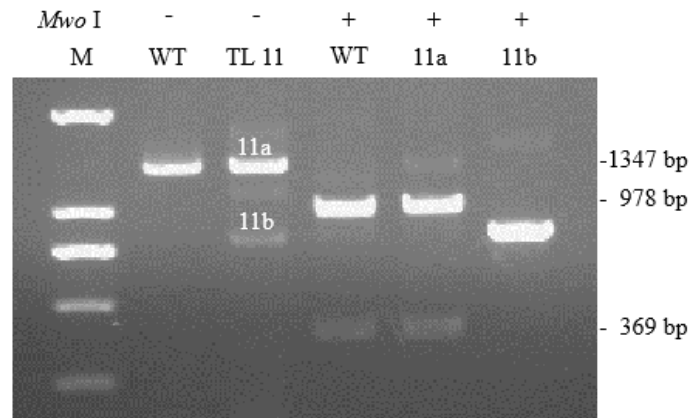


Figure S2 PCR/ restriction endonuclease assay to detect CRISPR/Cas9 genome edited mutations in the 1st generation of line 11. M, 2 kb DNA marker. -, undigested PCR products. +, digested PCR products. WT, PCR product of wild type hairy root of *M. truncatula*. TL11, PCR product of transgenic hairy root line 11. 11B, PCR product of the top band of transgenic hairy root line 11 purified by gel extraction kit. 11S, PCR product of the middle band of transgenic hairy root line 11 purified by gel extraction kit digested.

Fig S3

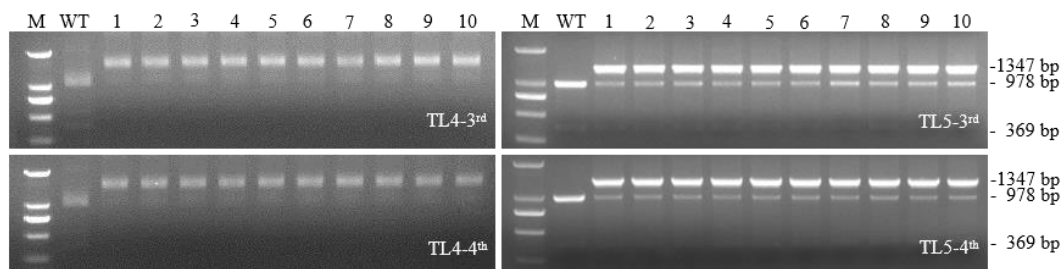


Figure S3 PCR/restriction endonuclease assay to identify CRISPR/Cas9 genome edited mutations in the 3rd and 4th generations of transgenic hairy root lines 4 and 5. PCR products amplified from transgenic hairy root line 4 and 5 digested with *Mwo* I. M, 2 kb DNA marker. WT, digested PCR product of wild type hairy root of *M. truncatula*; the 1,347 bp band corresponding to *MtPDS* was cut into two bands, 978 bp and 360 bp in size, by the *Mwo* I restriction endonuclease. TL4-3rd, the third generation of TL4. TL4-4th, the fourth generation of TL4. TL5-3rd, the third

generation of TL5. TL5-4th, the fourth generation of TL5. 1-10, digested PCR products of independent transgenic hairy roots from the 3rd and 4th generation of TL4 and TL5. No biallelic or homozygous hairy root mutation lines were found.

Fig. S4

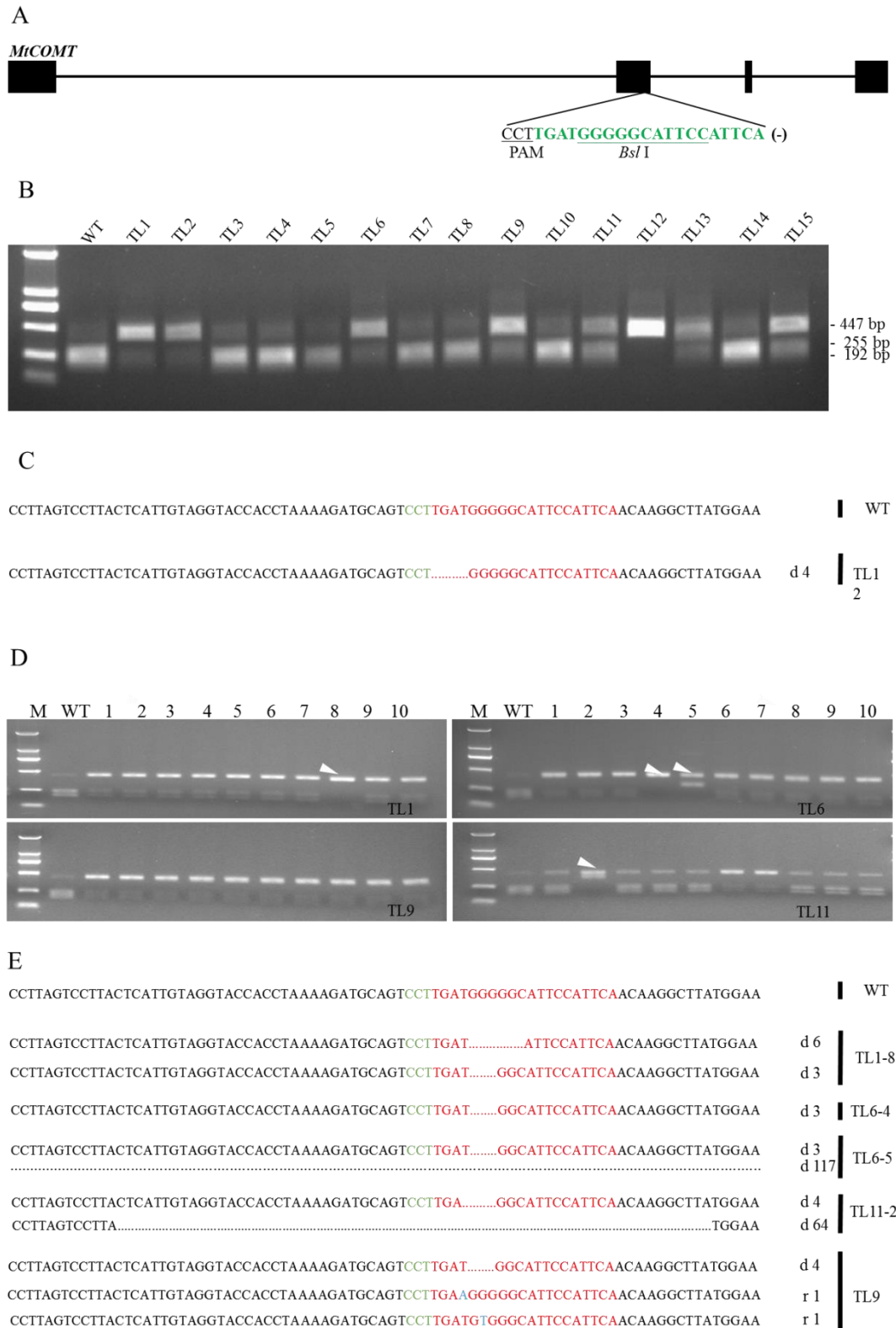


Figure S4 Screening for biallelic or homozygous mutated lines of *MtCOMT* in the 1st and 2nd generation of hairy root lines. (A) Schematic of the *MtCOMT* gene and the

selected target sequence. The nucleotides in green font were the target site in which the *Bsl* I recognition site were marked with blue lines, and the black font indicates the PAM. (B) PCR/restriction endonuclease assay to identify homozygous/biallelic lines for CRISPR/Cas9 genome edited mutations. Genotyping results for twenty 1st generation hairy root lines are shown. The 447 bp band from amplification of *MtCOMT* was cut into two bands 255 bp and 192 bp in size by the *Bsl* I restriction endonuclease. Lines 1-15, PCR products of co-transformed hairy root lines digested with *Bsl* I. Line 12 was biallelic for a mutation in *MtCOMT*; the 447 bp band could not be digested. The PCR products in the other lanes were amplified from chimeric or heterozygous mutated hairy root lines; the 447 bp band was partially digested by *Bsl* I. M, 2 kb DNA marker. WT, digested PCR product amplified from the wild-type control. (C) The sequence of *MtCOMT* of untransformed hairy root and types of mutations in TL12 in the first generation. Red bases indicate the target sequence; Green bases indicate the PAM (color figure online). The deletion is indicated by a dashed lines. d#, number of bases deleted from the target site. (D) Identification of five biallelic or homozygous hairy root mutation lines in the 2nd generation of lines 1, 2, 6, 9, and 15. DNA samples from independent lines were analyzed for mutations using the PCR/restriction endonuclease assay. M, 2 kb DNA maker. WT, digested wild-type control; the 447 bp band corresponding to *MtCOMT* was cut into two bands, 255 bp and 192 bp in size, by the *Bsl* I restriction endonuclease. 1-10, digested PCR products of transgenic hairy roots from the 2nd generation of TL1, TL2, TL6, TL9, and TL15 with *Bsl* I. White arrowheads indicate the bands amplified from biallelic or homozygous hairy root mutation lines. (E) Types of mutations in the biallelic or homozygous hairy root mutation lines screened from the 2nd generation of hairy roots and mutation types of 1st generation hairy root of TL9. Red and green bases indicate the target sequence and PAM region, respectively. Deletions are indicated by dashed lines. d#, number of bases deleted from the target site. r#, number of bases replaced at the target site.

Fig S5

GCAGATGCTGGTCAACAAGCCTATATTGCTGGAGGCAAGAGACGTTCTAGGTGGAAAGGTTTCTGACTAATT i1 | TL13
GCAGATGCTGGTCAACAAGCCTATATTG.....GAGGCAAGAGACGTTCTAGGTGGAAAGGTTTCTGACTAATT d3 | TL6-7

Figure S5 Targeted genome editing on *MtPDS* gene of continuous subculture generations of the two homozygous mutations found in line 13 and line 6. The mutation types of the two homozygous mutant lines were unchanged in the continuous subculture generations.

Table S1 The hairy root induction efficiency using different *A. rhizogenes* strains

<i>A. rhizogenes</i>	Total number of explants	Induction efficiency
LBA9402	89	67.7%±6.9%
A4	76	57%±6.7%
R1601	93	61%±5.1%
ATCC15834	91	53%±5.8%

Value are mean ± SD (n=3). The data were obtained as a mean of three replications. 33 explants were used in each test, and a total of 99 explants were used. After removing the explants of bacterial , the total number of rest explants were showed in the second column. The induction efficiency was counted as the proportion of explants induced hairy roots to total explants 20 days after infection.

Table S2 List of the primers used in this study.

Gene name	Primer sequence
<i>MtPDS</i> -tRNA-CRISPR-35S-B-1	TAGGTCTCAGTCAAACAAAGCACCAGTG
<i>MtPDS</i> -tRNA-CRISPR-sgRNA-2	GCGGTCTCAGGCTTGTGACCATGCACCAGC CGGGAA
<i>MtPDS</i> -tRNA-CRISPR-sgRNA-3	TAGGTCTCAAGCCTATATTGCGTTTTAGAG CTAGAAA
<i>MtPDS</i> -tRNA-CRISPR-35S-B-4	TAGGTCTCAAAACAAAAAAGCACCGACT CGGTGCC
<i>bar-F</i>	AGTCGACCGTGACGTCTCC

<i>bar</i> -R	GAAGTCCAGCTGCCAGAAAC
<i>rolB</i> -F	AAGTGCTGAAGGAACAATC
<i>rolB</i> -R	CAAGTGAATGAACAAGGAAC
<i>Cas9</i> -F	ATCCAAGCGAAACGGGAGTT
<i>Cas9</i> -R	ACCGCCACTCCATCAAGAAG
<i>virG</i> -F	CCTTGGGCGTCGTCATAC
<i>virG</i> -R	TCGTCCTCGGTCGTTTCC
<i>MtPDS</i> -F	GATTATCCACGTCCTGAGCT
<i>MtPDS</i> -R	TGTAGGCCGGTCTCATACCA
<i>Cas9</i> -qRT-F	GCCGCTCTGCTTATCCCT
<i>Cas9</i> -qRT-R	ACATCGTCCTCACTCTTACTCTCT
<i>MtActin</i> -qRT-F	CCACATGCCATCCTTCGTTT
<i>MtActin</i> -qRT-R	TGTCACGGACAATTTCCCG

Red letters and dark blue letters indicate the recognition sequences and cutting sites of *Bsa* I, respectively; light blue letters indicate the target sequences in *MtPDS*.

Table S3 The sequence of pYLCRISPR/Cas9 35S-B-AtU3b

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AtU3b Promoter

The two BsaI cutting sites

SgRNA sequence