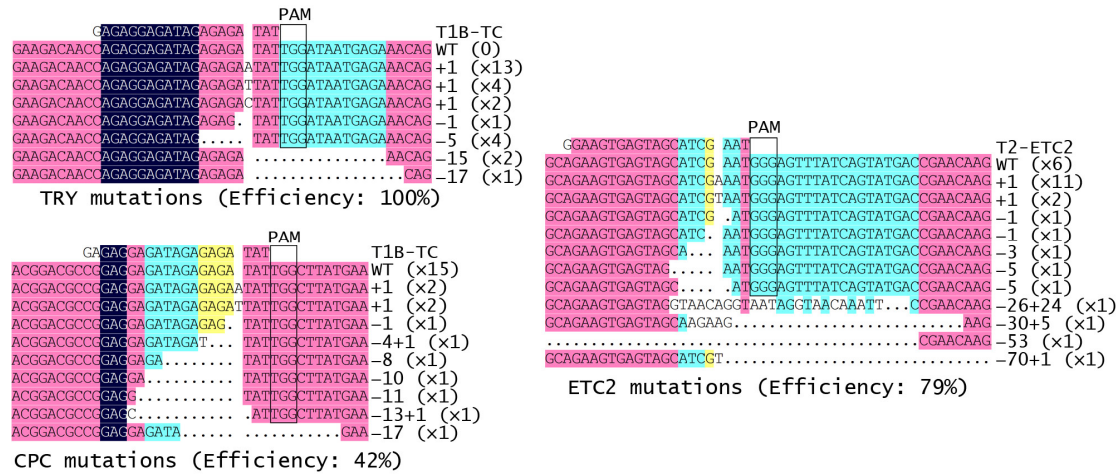


## Additional file 1

**Figure S1**



**Figure S1** Sequencing analysis of target gene mutations of a representative p2gR-TRI-B Line.

**Table S1**

**Table S1** Primers used in this study

Primer name	Primer sequence (5'→3')
<b>Primers for construction of gRNA expression cassettes and premade gRNA modules</b>	
U6-26-HiF	TCAAGCTTCGACTTGCCTCCGCACAATACATC
U6-26-HiR	ATCAAGCTTATTGGTTTATCTCATCGGAACTGC
U6-1-HiF	TAAGCTTGAGACGGTCATTGTTTTAGTTCCAC
U6-1-HiR	CAAGCTTAACGGACCAATCACTTTGTCTTAGC
U6-29-HiF	TACAAGCTTAATCCAAACTACTGCAGCCTGAC
U6-29-HiR	TCAAGCTTCATCATATGTCCTCATTCCCTGTC
OsU3-HiF	ATATCAAGCTTAGTAATTCATCCAGGTCTCCAAGTT
OsU3-HiR	AATTCAAGCTTAGCAGCAAGCAGTATCGATCACACC
TaU3-HiF	ATATCAAGCTTCATGAATCCAAACCACACGGAGTTC
TaU3-HiR	ATTATGAAGCTTGTTCCGGCTGTCTTGTCCACAGAG
Sp-gR-BsF	TGTGAGACCAACCCAGTGGACATAAGC
Sp-gR-R01	AGCCTTATTTAACTTGCTATTTCTAGCTCTAAAACCGAGACCGGCTTATTATGCACGCT
Sp-gR-R02	AAGCACCAGCTCGGTGCCACTTTTTCAAGTTGATAACGGACTAGCCTTATTTAACTTGCTATTTCTAGC
Sp-gR-DrR	TCTATCTTTAAAAGCACCGACTCGGTGCCACTT
U6-26-hDrF	TTTTGCAAAAATTTCCAGATCGATTTCTTCTTCCCTC
U6-26-EcR	TATTAGATACACTACTTCGACTCTAGCTGTAT

U61-hDrF	TTTTGGCAAAAATTTTCAGATTTTTTCTTCATCTGTAGATTTCTGG
U61-EcR	ATTCAGATATCACTACTTCGTCTCTAACCAT
U6-29-hDrF	TTTTGGATAGAATTTCCAGCTTTTTTTCGCTGTTTCAGC
U6-29-EcR	ATATTAGATATCTCTTAGTCGACTCTACCAAT
OsU3D-NBF	TATATTACCATGGCAGTGACTGGTCTCATT
OsU3D-F0	CCATGGCAGTGACTGGTCTCATTTTTTTTTTTCGTTTTGCATTGAGTTTTCTCCGTCGC
OsU3D-NBR	ATTTATCCATGGTCTCAGCCACGGATCATCTGCACAACCTCTT
Sp-gR-BBF	ATAATAACGTCTCTTGGCGTGAGACCAACCCAGT
Sp-gR-BsR	AATATTACGTCTCTAAAAGCACCGACTCGGTGCCACTT
TU3D-NBF	ATTACTTACCATGGACATACCTGCAACATT
TU3D-F0	TACCATGGACATACCTGCAACATTTTTTTTTTGCTTCTGTTTTTTAGTCAGTCTC
TU3D-NBR	ATTATATTCCATGGTACCTGCTCGTGCTTCTTGGTGCC
Sp-gR-BBF3	ATAATAACGTCTCTAAGCGTGAGACCAACCCAGT
DT1F0	TGGACGGCCATGGCGGTTCTCGTTTTAGAGCTAGAAATAGC
Ter1Pro2-R	GTCAGGCTGCAGTAGTTGGATTAATATTGGTTTATCTCATCGGAACTGC
Ter1Pro2-F	GCAGTTCGATGAGATAAACCAATATTAATCCAACTACTGCAGCCTGAC
DT2R0	AACTGCCATTGTATAACTTTGCCAATCTCTTAGTCGACTCTAC
DT1F	AATAATGGTCTCTATTGGACGGCCATGGCGGTTCTCGTT
DT2R	ATTATTGGTCTCTAAACTGCCATTGTATAACTTTGC
DT2F0	TGGCAAAGTTATACAATGGCAGTTTTAGAGCTAGAAATAGC
Ter2Pro3-R	GTGGAATAAACCAATGACCGTCTCATATATGCCTCATTCCCTGTC
Ter2Pro3-F	GACAGGAATGAGGACATATGATGAGACGGTCATTGTTTTAGTTCCAC
DT3R0	AACGATCTAGATTTCCCGTAACAATCACTACTTCGTCTCTAACCAT
DT2F	AATAATGGTCTCTATTGGCAAAGTTATACAATGGCAGTT
DT3R	ATTATTGGTCTCTAAACGATCTAGATTTCCCGTAAC
DT3F0	TGTTACCGGGAAATCTAGATCGTTTTAGAGCTAGAAATAGC
Ter3Pro1-R	GATGTATTGTGCGGAAGCAAGTCAACGGACCAATCACTTTGTCTTAGC
Ter3Pro1-F	GCTAAGACAAAGTGATTGGTCCGTTGACTTGCCTCCGCACAATACATC
DT4R0	AACCATCTCCATATCTCTTCTCAATCACTACTTCGACTCTAGCTGTAT
DT3F	AATAATGGTCTCTATTGTTACCGGGAAATCTAGATCGTT
DT4R	ATTATTGGTCTCTAAACCATCTTCCATATCTCTTCT
MT-F	GTTTTAGAGCTAGAAATAGCAAGTT
MTer1Pro2-R0	GAACCCGTGTGGTTTGGATTATGAGCAGCAAGCAGTATCGATCACACC
MTer1Pro2-F0	GGTGTGATCGATACTGCTTGTGCTCATGAATCCAAACCACAGGAGTTC
MT2-R	GGCATGCTCCTCTGGTACGCTTCTTGGTGCC
MTer2Pro3-R0	GATGTATTGTGCGGAAGCAAGTCTGTTCCGGCTGTCTTGTACAGAG
MTer2Pro3-F0	CTCTGTGACAAGACAGCCGAACACGACTTGCCTCCGCACAATACATC
MT3-R	TACTACTTTCGACTCTAGCTGTAT
MTer3Pro4-R0	AACTGGTGACCTGGATGAATTACTTATTGGTTTATCTCATCGGAACTGC
MTer3Pro4-F0	GCAGTTCGATGAGATAAACCAATAAGTAATTCATCCAGGTCACCAAGTT
MT4-R	CGCCACGGATCATCTGCACAACCTCTT
<b>Primers for construction of pGreen-like binary vectors</b>	
pSa-ori-XbF	ATTATTTCTAGATCCCCATCCAACAGCCCGC

pSa-ori-XbR	ATTATTCTAGACCATGAATCCAGAAGCCCGAGAGGT
Marker-EcF	ATTTAGAATTCGAGGCGGTTTGCCTATTGGCTAGAG
Marker-SpR	ATTTAACTAGTGACAACCTAATAACACATTGCGGAC
<b>Primers for construction of the plant CRISPR/Cas9 vector set</b>	
oXX-F	TGTATGGCCGCGGCCGCACCGGTAGGCTCCATACAA
oXX-R	TGTATGGAGGCCTACCGGTGCGGCCGCGCCATACAA
<b>Primers for construction of one-gRNA-expressing vectors for gene targeting</b>	
Oligo-01F/ZT1	ATTGGCTTCGTGCCAACCAACGA
Oligo-R/ZT1	AAACTCGTTGGTTGGCACGAAGC
Oligo-11F/ZT1	GGCGGCTTCGTGCCAACCAACGA
Oligo-21F/ZT1	AGCGGCTTCGTGCCAACCAACGA
<b>Primers for construction of two-gRNA-expressing vectors for gene targeting</b>	
MT1-BsF/ZH	AATAATGGTCTCTGGCGGCTTCGTGCCAACCAACGA
MT1-F0/ZH	GGCTTCGTGCCAACCAACGAGTTTTAGAGCTAGAAATAGC
MT2-R0/ZH	GGCATGCTCCTCCTGGTCACGCTTCTGGTGCC
MT2-BsR/ZH	ATTATTGGTCTCTAAACGGCATGCTCCTCCTGGTCA
DT1A-BsF/TC	AATAATGGTCTCTATTGAATATCTCTATCTCCTCGTT
DT1A-F0/TC	TGAATATCTCTATCTCCTCGTTTTAGAGCTAGAAATAGC
DT2-R0/ETC2	AACATTGATGCTACTCACTTCCAATCTCTTAGTCGACTCTAC
DT2-BsR/ETC2	ATTATTGGTCTCTAAACATTTCGATGCTACTCACTTC
DT1B-BsF/TC	AATAATGGTCTCTATTGAGAGGAGATAGAGAGATATGTT
DT1B-F0/TC	TGAGAGGAGATAGAGAGATATGTTTTAGAGCTAGAAATAGC
DT1-BsF/CHLI1	AATAATGGTCTCTATTG-CCCCATTTGCTTCAGGCCGTT
DT1-F0/CHLI1	CCCCATTTGCTTCAGGCC-GTTTTAGAGCTAGAAATAGC
DT2-R0/CHLI2	TGTCTCTGTTATGAATGTCCAATCTCTTAGTCGACTCTAC
DT2-BsR/CHLI2	ATTATTGGTCTCTAAACTGTCTCTGTTATGAATGTC
<b>Primers for analysis on target gene mutations</b>	
ZT-IDF0	AAGGACGGCCATGGCGATTTTCGCAT
ZT-IDR0	CCGATGATCTTCTGATAGATGGATC
ZT-IDF	TATCGTGCTGGGCTACATCTTGGTC
ZT-IDR	CGGAGTGCCTGGAGTTGACGGACAT
TRY-IDF	ATGTACAGACTTGTCGGTGATAGGT
TRY-IDR	GGACGGTGAGGCTTGGTATGTTTGT
CPC-IDF	GGTCTAACTTACCGAGCTGTCAATG
CPC-IDR	CAAAATAGTAATTCAAGGACAGGTACAT
CPC-off-IDF	GTTTCGTGTCTTCAGATTAGTTCGATGT
CPC-off-IDR	TCATTGACAGCTCGGTAAGTTAGAC
ETC2-IDF	CAGTAGTTATGGATAATACCAACCGTCT
ETC2-IDR	ATCAGCTTTGATTTGTTACTCTCGCCAT
TRY-IDF0	ATGTACAGACTTGTCGGTGATAGGT
TRY-IDR0	GTCTCATGGATTTCGTTGTATAGCGT
TRY-SeqF	CATGTATATAAATGCTTGGCTGGCTCAA
CPC-IDF0	GGTCTAACTTACCGAGCTGTCAATG

CPC-IDR0	GAGCTACCTCGTTGACCCATATCGT
CPC-SeqF	TAATTTATGTCAGAACTCACTTTGGCTA
ETC2-IDF0	CCTCACATCTTTTCTATATCTTGCATT
ETC2-IDR0	TTCCTGCTATTAATCCACCTGAC
ETC2-SeqF	GTTCACTCGATCCCGATATGACTCT
<b>Primers for counterselection of nontransgenic T2 plants</b>	
Hyg-IDF1	CCCGATTCCGGAAGTGCTTGACATT
Hyg-IDR1	CGCTCCAGTCAATGACCGCTGTTAT
Hyg-IDF2	CAAAGATCGTTATGTTTATCGGCACT
Hyg-IDR2	AAGAAGATGTTGGCGACCTCGTATT
zCAS9-IDF	CGGCCTCGATATTGGGACTAACTCT
zCAS9-IDR	CTTATCTGTGGAGTCCACGAGCTTC