Table S1. Primers used for vectors construction, mutation detection, off-target analysis and qPCR.

FI GTGTGGTGTTGTTGCTGCTCATGTTT R1 CCAGCGCCCAATCATTTACCCCTC F2 AGTTGTTTGAATACTATGACCAGGATCTCCC R2 GCAGCATATACGCGGAAAGAATTATAAACGAT crispr-gRNA1-F GGTCTCTgattCAGGTCGTCCCATCAAGATGTTTAGAGCTAGAAATAG crispr-gRNA2-R GGTCTCTAAACCTAAGCCAGTATCAGACTCCAATCACTACTTCGTCTCTA crispr-gRNA3-F GGTCTCTAAACCTAAGCCAGTATCAGACTCCAATCACTACTTCGTCTCTA prg-gRNA1-F GGTCTCTAAACCGACGTCTGTCCCATCAAGATGTTTCAGAGCTAGGAAATAG crispr-gRNA4-R GGTCTCTAAACGTCTTTAACAGTTAAACCATAATCACTACTTCGTCTCTA prg-gRNA1-F GGTCTCTAAACCGACGGTCTGTCCCATCAAGATGTTTCAGAGCTATGCTGGA prg-gRNA3-F GGTCTCTAAACCTAGCAGGAGAGAGAGAGCAGCCGGGAATCGA prg-gRNA4-R GGTCTCTAAACGTCTTTAACAGTTAACACTTGCACCAGCCGGGAATCGA prg-gRNA4-R GGTCTCTAAACGTCTTTAACAGTTAACACTTGCACCAGCCGGGAATCGA SP-DL GCCGGACATAGATGCAATAACTTC GF aGAGACCGGTCTCGGTTTCAGAGCTATGCTGGAAACAGC GR AGCTCGAGAGGCGCGAAAAAAGCACCGACTCGGTCCCACCATTTTCAAGGTGAT U6-1-F GACCGGTACTGAGAGCGGGAAAATCCAAAATTCGCTCGACCAGACAA U6-1-F CACTGGGGCTCTGTAACACATACTTCGTCTCTAACCATATAT U6-1-R CACTGGGGCCCAGGGGCCAAACACTCGTTCGAACCATGAACCCGGGTCTGTAACGAGCCCGGGGCGGAACAACCCGGGCTCGCAC gRNA-R GCACCGACTCGGTGCCAC
R1CCAGCGCCCAATCATTACCCTCF2AGTGTTTGAATACTATGACCAGGATCTCCCR2GCAGCATATACGCGGAAAGAATTATAAACGATcrispr-gRNAFGGTCCTGattCAGGTCGTCCCATCAAGATGTTTAGAGCTAGAAATAGcrispr-gRNAFGGTCCTTGAAACCTAAGCCAGTATAGACTCCAATCACTACTTCGTCTCAAcrispr-gRNAFGGTCCTTGCAACGTCTTAACAGTATAAGCATATAGTTTAGAGCTAGAAATAGpl-gRNAFGGTCCTTGCAACGTCTTAACAGTACAACATAACCATACTCGTGCCATGCGGApl-gRNAFGGTCCTTGCAACGTCTGCCATCAAGATCAGACCAGCGGGAATCGAGpl-gRNAFGGTCCTTAGCAACTCGAGGATGATAAGTTTCAGAGCTATGCTGGApl-gRNAFGGTCCTAAACGTCTTAACAGTAACCATAGCTGCGCAGCAGGGAATCGApl-gRNAFGGTCCTCAAACGTCTTGACCGGSP-DLGTCGTGCCCACATGTTGACCGGSP-RCCCGACATAGATGCAGTATGCTGGAAACAGCGRAGACCGGTCTCGGTTCAAGACTAGCGGAGAACAGCGFGAGACCGGTCTCGATTCAGAGCACAGCAGCAGAACAAU6-1-FGAGACCGGTCTGAATCACTACTTCGTCTCTAACCATATATU6-1-RCACCGGTACGGGGAGAAAAACCACGAGCAGAACAAAAGCACGGAGAACAAAU6-1-RCACCGGTCTGTAATCACTACTTCGTCTCTAACCATATATTFAACAAAGCACCAGTGGTCTAGTGGTAGAATAGTACCTGGCAGAGACAGAGCgRNAFGGAGCTGCTCCACAGAGGTTCAGAGCCGGGAATCGAACCGGGTCTGTACCGTGGgRNAFCACGGACTGGGTCAGGAGATTCCAAGACgRNAFGGGTCTAACGTTAAGGCTTAGGGTTCAGAGCgRNAFGAGACTGGTGTAAAGACGTTCAGAGCgRNAFGAGACTGGTGAGAGAGAGAGAGACACTGAATGGTGTAAAGACGTTCAGAGCgRNAFGAGACTGGTGAGAGAGAGAGAGCgRNAFGAGACTGGTGTAAGGCTAGGGTCAGAGACgRNAFGAGACTGGTGAGAGAGAGAGAGCgRNAFGAGACTGGTGAGAGAGAGAGAGCGTTCAGAGCgRNAFGAGACTGGAGAGAGAGAGAGGAGAGAGAGAGAG
F2AGTTGTTGAATACTATGACCAGGATCTCCCR2GCAGCATATACGCGGAAAGAATTATAAACGATcrispr-gRNAI-FGGTCTTgattCAGGTCTGTCCCATCAAGATGTTTTAGAGCTAGAAATAGcrispr-gRNA2-RGGTCTTAAACCTAAGCCAGTAGATATAGTTTTAGAGCTAGAAATAGcrispr-gRNA3-FGGTCTTGAAACGAGGTCTGTCCATCAAGAATTAGTTTAGAGCTAGACATGGAptg-gRNA1-FGGTCTTGAAACGTCTTAACAGTACAGACTCTGCACCAGGCAGG
R2GCAGCATATACGCGGAAAGAATTATAAACGATcrisp-gRNA1-GCTCTCgattCAGGCTGTCCCATCAAGATGTTTAGAGCTAGAAATAGcrisp-gRNA2-GTCTCTAAACCTAAGCCAGTATCAGACTCCAATCACTACTTCGTCTCAAcrisp-gRNA1-GTCTCTGAACGTCTTTAACAGTTAAACCATAATCACTACTTCGTCTCAApt-gRNA1-GTCTCTGCAACAGGTCTGTCCCATCAAGATGTTTAGAGCTAGGGGAATCGApt-gRNA1-GTCTCTGCAACGGTCTGTCCCATCAAGACGCGGGGAATCGApt-gRNA1-GTCTCTGCAACGGCTGTGCCAGTAGAGATATAGTTTCAGAGCTAGCGGGAATCGApt-gRNA1-GTCTCTGCAACCGAGTAGATGATAAGTTTCAGAGCTAGCGGGAATCGApt-gRNA1-GTCTGTGCACCAGTTTAACAGTAAACCATTGCACAGCGGGAATCGApt-gRNA4-GTCCTGCACCAGTTTAACAGTAAACCATTGCACAGCCGGGAATCGApt-gRNA4-GTCGTGCTCCACAAGTTGACAGGpt-gRNA4-GTCCTGGACTCGATTGACAGGTAGATGATAGTTTCAGAGCTATGCTGGApt-gRNA4-GGTCCTGAACGGCTTGAAACACACAGCGSP-DLGTCGTGCCCCACAGTTGACAGGCCAGAAAAAGCACCGAGCACAACAGCGFGAGACCGGTCTCGATTAGAGCACCGAGCAGAACAACACGFGAGACCGGTCTCGATAACATCTCAAAATTCCGGCAGAACAAU6-1-CGAGACCGGTCTCGATCAACATCTCGTCTAACCATATATU6-1-RACCGGATGGTGTAATCACTACTTCGTCTAACCATATATTFAACAAAGCACCAGGGTCTCAGTGTAGAATAGTACCTGGCAGCAGGACTGAACAGACCGGGTTRACCGACCGGTCCCACAAGATGTTCAGAGCACCGGGTCTGTACCGTGGRNA1-RGAGCTGTGTCCAATCAGAGTTTCAGAGCgRNA4-RGAGCTGGTGCACAGGTTAGAGTTCAGAGCgRNA4-RACCGATGGATGATATAGTTCAGAGCgRNA4-RACCGATGGATGATATAGTTCAGAGCgRNA4-RACCGATGGATGATATAGTTCAGAGCgRNA4-RACCGATGGATGATATAGTTCAGAGCgRNA4-RACCGATGGATGATATAGTTCAGAGCgRNA4-RACGGATGGAGAGAGATGATATAGTTCAGAGC
crispr-gRNA1-FGGTCTCTgattCAGGTCTGTCCCATCAAGATGTTTTAGAGCTAGAAATAGcrispr-gRNA2-RGGTCTCTAAACCTAAGCCAGTATCAGACTCCAATCACTACTTCGTCTCTAcrispr-gRNA3-FGGTCTCTgattACTCGATGGATGATGATAAACCATAATCACTACTTCGTCTCTAptg-gRNA1-FGGTCTCTTGCACAGGTCTGTCCCATCAAGATGTTTCAGAGCTATGCTGGAptg-gRNA2-RGGTCTCTTGCACAGGTCTGTCCCATCAAGATGTTTCAGAGCTATGCTGGAptg-gRNA3-FGGTCTCTTGCACACGCAGTGGATGATAACCATAGTTTCAGAGCTATGCTGGAptg-gRNA3-FGGTCTCTAAACCTCGATGGATGATGATATAGTTTCAGAGCTATGCTGGAptg-gRNA3-FGGTCTCTAAACGTCTTTAACAGTAAACCATTGCACCAGCCGGGAATCGAptg-gRNA3-FGGTCTCTGAACGCGATGGATGATGATATAGTTTCAGAGCTATGCTGGAptg-gRNA4-RGGTCTCTGAACGCGGTCTCGGGTACAGGGSP-DLGTCGTGCCCACATGTTGACCGGGFaGAGACCGGTCTCGGTTTCAGAGCTATGCTGGAAACAGCGFaGAGACCGGTCTCGGTTTCAGAGCTATGCTGGAAACAGCGRAGCTCGAGAGGCGCGAAAAAAGCACCGACTCGGTGCCACTTTTCAAGTTGATU6-1-FGACCGGTCTCTGAATCACTACTTCGTCTCTAACCATATATU6-1-RCACTGGTGCTTGTAATCACTACTTCGTCTTAACCATATATTFAACAAAGCACCAGTGGTCTAGTGTAGGATAGAACCGGGCTCGTACAGGCCGGGGTgRNA-RGGAGTCTGTCCCATCAAGATGTTTCAGAGCgRNA-RGGAGTCTGATACTGGCTTAGGTTTCAGAGCgRNA-FAGGTCTGATACTGGCTTAGGTTTCAGAGCgRNA-FAGGTCTGATACTGGTTAAGATATAGTTCAGAGCgRNA-FAGGTCTGATACTGGTTAAGACGTTTCAGAGCgRNA-FAGGTTTAACTGTTAAAGACGTTTCAGAGCgRNA-FAGGTTTAACTGTTAAAGACGTTTCAGAGCgRNA-FAGGTTTAACTGTTAAAGACGTTCAGAGCgRNA-FAGGTCTGATACTGGTGCCAGAAGATgRNA-FAGGTTGAAACTGGTTGCCAGAGATTCCAGAGCgRNA-FAGGTTGAAACTGGTTAGA
crispr-gRNA2-RGGTCTCTAAACCTAAGCCAGTATCAGACTCCAATCACTACTTCGTCTCTAcrispr-gRNA3-FGGTCTCTgattACTCGATGGATGATGATATAGTTTAGAGCTAGAAATAGcrispr-gRNA4-RGGTCTCTGCACAGGTCTGTCCCATCAAGATGTTTCAGAGCTAGCT
crispr-gRNA3-FGGTCTCTgattACTCGATGGATGATGATATAGTTTTAGAGCTAGAAATAGcrispr-gRNA4-RGGTCTCTAAACGTCTTTAACAGTTAAACCATAATCACTACTTCGTCTCTAptg-gRNA1-FGGTCTCTTGCACAGGTCTGTCCCATCAAGATGTTTCAGAGCTATGCTGGAptg-gRNA2-RGGTCTCTAAACCTAAGCCAGTAGAGATGATATAGTTTCAGAGCTATGCTGGAptg-gRNA3-FGGTCTCTAAACGTCTTTAACAGTTAAACCATGCAGCAGCGGGAATCGAptg-gRNA4-RGGTCTCTAAACGTCTTTAACAGTTAAACCATTGCACCAGCCGGGAATCGASP-DLGTCGTGCTCCACATGTTGACCGGSP-NCCCGACATAGATGCAATAACTTCGFaGAGACCGGTCTCGGTTCAGAGCAGCAGCAGCAGCAGCAGCAGAACAGCGRAGCTCGAGAGGCGCGAAAAAAGCACCGACTCGGTGCCACTTTTTCAAGTTGATU6-1-FGACCGGTCTCtAATCACTACTTCGTCTCTAACCATATATU6-1-RCACTGGTGCTTGTAATCACTACTTCGTCTCTAACCATATATTFAACAAAGCACCAGTGGTCTAGTGGTAGAATAGTACCAGGCGGACAACAATRCGAGACCGGTCTCtGACCAGCCGGGAAATGAACCGGGCTCGTACCGGGGgRNA-RGCACCGACTCGGTGCCACgRNA-FCAGGTCTGTCCATCAAGAGTTTCAGAGCgRNA-FACTCGATGGATGATGATATAGTTTCAGAGCgRNA-FACTCGATGGATGATGATATAGTTCAGAGCgRNA-FACGAGGATGCGTAGAGATGATATAGTTCAGAGCgRNA-FACTCGATGGATGATGATATAGTTCAGAGCgRNA-FACGGTCTGATACTGGCTAGGTTCAGAGCgRNA-FACGGTTGATACTGTTAAAGACGTTCCAGAGCgRNA-FACGAGATTCCGTTGCCAGAAGTACTCGATGGATGATGATATAGTTCAGAGCgRNA-FTGGTTAACTGTTAAAGACGTTCCAGAGCgRNA-FTGGTTAACTGTTAAAGACGTTCCAGAGCgRNA-FTGGTTAACTGTTAAAGACGTTCCAGAGCgRNA-FTGGTTAACTGTTAAAGACGTTCAGAGCgRNA-FTGGAGATTCCGTGCCAGAAAGTgRNA-FTGGAGATCCGTTGCCAGAAG
crispr-gRNA4-RGGTCTCTAAACGTCTTTAACAGTTAAACCATAATCACTACTTCGTCTCTAptg-gRNA1-FGGTCTCTTGCACAGGTCTGTCCCATCAAGATGTTTCAGAGCTATGCTGGAptg-gRNA2-RGGTCTCTAAACCTAAGCCAGTGGATGATGATATAGTTTCAGAGCTATGCTGGAptg-gRNA4-RGGTCTCTAAACGTCTTTAACAGTTAAACCATTGCACCAGCCGGGAATCGAptg-gRNA4-RGGTCTCTAAACGTCTTTAACAGTTAAACCATTGCACCAGCCGGGAATCGASP-DLGTCGTGCTCCACATGTTGACCGGSP-RCCCGACATAGATGCAATAACTTCGFaGAGACCGGTCTCGGTTTCAGAGCACACGCGCAGAACAGCGRAGCTCGAGAGGCGCGAAAAAAGCACCGACTCGGTGCCACTTTTCAAGTTGATU6-1-FGACCGGTCTCtAATCACTACTTCGTCTCTAACCATATATU6-1-RCACTGGTGCTTTGTAATCACTACTTCGTCTCTAACCATATATTFAACAAAGCACCAGTGGTCTAGTGGTAGAATAGTACCCTGCCACGGTACAGACCCGGGTTRCGAGACCGGTCTCtTACACAGCCGGGAATCGAACCCGGGTCTGTACCGTGGgRNA-RGCACCGACTCGGTGCCACgRNA1-FCAGGTCTGATACTGGCTTAGGTTCCAGAGCgRNA3-FACTCGATGGATGATATAGTTCCAGAGCgRNA3-FACTCGATGGATGATATAGTTCCAGAGCgRNA4-FATGGTTTAACTGTTAAAGACGTTTCAGAGCgRNA4-FATGGTTTAACTGTTAAAGACGTTCAGAGCgRNA4-FATGGTTTAACTGTTAAAGACGTTCAGAGCgRNA4-FATGGTTTAACTGTTAAAGACGTTCAGAGCgRNA4-FATGGTTTAACTGTTAAAGACGTTCAGAGCgRNA4-FATGGTTTAACTGTTAAAGACGTTCAGAGCgRNA4-FATGGTTTAACTGTTAAAGACGTTCAGAGCgRNA4-FATGGTTTAACTGTTAAAGACGTTCAGAGCgRNA4-FATGGTTTAACTGTTAAAGACGTTCAGAGCgRNA4-FATGGTTAACTGTTAAAGACGTTCAGAGCgRNA4-FATGGTTAACTGTTAAAGACGTTCAGAGCgRNA4-FATGGTTAACTGTTAAAGACGTTCAGAGCgRNA4-FATGG
ptg-gRNA1-FGGTCTCTTGCACAGGTCTGTCCCATCAAGATGTTTCAGAGCTATGCTGGAptg-gRNA2-RGGTCTCTAAACCTAAGCCAGTATCAGACTCCTGCACCAGCCGGGAATCGAptg-gRNA3-FGGTCTCTGCAACTCGATGGATGATGATATAGTTTCAGAGCTATGCTGGAptg-gRNA4-RGGTCTCTAAACGTCTTTAACAGTTAAACCATTGCACCAGCCGGGAATCGASP-DLGTCGTGCTCCACATGTTGACCGGGFCCCGACATAGATGCAATAACTTCGFaGAGACCGGTCTCGGTTTCAGAGCTATGCTGGAAACAGCGRAGCTCGAGAGGCGCGAAAAAAGCACCGACTCGGTGCCACTTTTCAAGTTGATU6-1-RGACCGGTCTCAATCACTACTTCGTCTCTAACCATATATU6-1-RCCAGGACCGGTCTCAATCACTACTTCGTCTCTAACCATATATTFAACAAAGCACCAGTGGTCTAGTGGTAGAATAGTACCTGGCAACAGACCCGGGTTRCGAGACCGGTCTCTTACACAGCGGGAATCGAACCCGGGTCTGTACCGTGGgRNA-RGACCGACTCGGTGCCACgRNA-FGAGTCTGATACTGGCTAAGTTTCAGAGCgRNA3-FACTCGATGATGATGATATAGTTTCAGAGCgRNA3-FACTGATGGATGATAATAGTTCAGAGCgRNA4-FTGGTTTAACTGTTAAAGACGTTTCAGAGCgRNA4-FATGGTTTAACGTTAGCAGAAGCgRNA4-FATGGTTAACTGTTAAAGACGTTCAGAGCgRNA4-FATGGTTAACTGTTAAAGACGTTCAGAGCgRNA4-FTGAGAATCCGTGCCCAGAAGTACHD1181-FTCCTACTCATGGGTCTGCGAAT
ptg-gRNA2-RGGTCTCTAAACCTAAGCCAGTATCAGACTCCTGCACCAGCCGGGAATCGAptg-gRNA3-FGGTCTCTTGCAACTCGATGGATGATGATAAGTTTCAGAGCTATGCTGGAAptg-gRNA4-RGGTCTCTAAACGTCTTAACAGTTAAACCATTGCACCAGCCGGGAATCGASP-DLGTCGTGCTCCACATGTTGACCGGSP-RCCCGACATAGATGCAATAACTTCGFaGAGACCGGTCTCGGTTTCAGAGCTATGCTGGAAACAGCGRAGCTCGAGAGGCCGAAAAAGCACCGACTCGGTGCCACTTTTCAAGTTGATU6-1-FGACCGGTAAGGCGCGAAAATCTCAAAATTCCGGCAGAACAAU6-1-RCGAGACCGGTCTCAATCACTACTTCGTCTCAACCATATATTFAACAAAGCACCAGTGGTCTAGTGGTAGAATAGTACCTGGCAGGTACAGAGCCGGGATRCGAGACCGGTCTCTGCACCAGCGGAATCGAACCGGGTCTGTACCGGGTCTGTACCGGGTCTGTACCGGGTCTGTGTAATCACTAGTGTCAGAGCCGGGAATCGAACCGGGTCTGTGCACgRNA-RCGACCGACTCGGTGCCACgRNA2-FGGAGTCTGATACTGGCTTAGGTTCAGAGCgRNA3-FACCGATGGATGATGATATAGTTCAGAGCgRNA4-FAGGTTTAACTGTTAAAGACGTTTCAGAGCgRNA4-FGGAGATTCCGTTGCCCAGAAGTAcha107181-FGAGAGATTCCGTGCCACGAAGTAcha107181-FGAGAGATTCCGTGCGGTCTGCGAT
pig-gRN34-9GGTCTCTGCAACTCGATGGATGATGATAAGTTTCAGAGCTATGCTGGAApig-gRN44-8GGTCTCTAAACGTCTTTAACAGTTAAACCATTGCACCAGCCGGGAATCGASP-DLGTCGTGCTCCACATGTTGACCGGSP-RCCCGACATAGATGCAATAACTTCGFaGAGACCGGTCTCGGTTTCAGAGCTATGCTGGAAACAGCGRAGCTCGAGAGGCGCAAAAAAGCACCGACTCGGTGCCACTTTTCAAGTTGATU6-1-FGAGACCGGTCTCtAATCACTACTTCGTCTCTAACCATATATU6-1-RCACTGGTGCTTGTAATCACTACTTCGTCTCTAACCATATATU6-1-RAACAAAGCACCAGTGGTCTAGTGGTAGAATAGTACCTGGCAGGGAACAGACCGGGTTFAACAAAGCACCAGTGGTCTAGTGGTAGAATAGTACCTGGCACGGGTACAGACCCGGGTgRN4-RGCACCGACTCGGTGCCACgRN4-FGAGGTCTGATAGAGCGTTCAGAGCgRN4-FATCGATGGATGATAAGTTTCAGAGCgRN4-FATCGATGGATGATAAAGACGTTCAGAGCgRN4-FAGGTTTAACTGTTAAAGACGTTCAGAGCgRN4-FAGGTTTAACTGTTAAAGACGTTCAGAGCgRN4-FATGGTTTAACGTGTGCCAGAAGTgRN4-FATGGTTAACTGTGCCAGAAGTgRN4-FATGGTTAACGTTGCCAGAAGTgRN4-FATGGTTAACGTGCCAGAAGTgRN4-FATGGTTAACTGTGCCAGAAGTgRN4-FATGGTTAACGTGCCAGAAGTgRN4-FATGGTTAACGTGCCAGAAGTgRN4-FATGGTTAACGTGCCAGAAGTgRN4-FATGGTTAACTGTGCCAGAAGTgRN4-FATGGTTAACGTGCCAGAAGTgRN4-FATGGTTAACGTGCCAGAAGTgRN4-FATGGTTAACGTGCCAGAAGTgRN4-FATGGTTAACTGCGTGCCAGAAGTgRN4-FATGGTTAACTGCGTGCCAGAAGTgRN4-FATGGTTAACTGCGTGCCAGAAGTgRN4-FATGGTTAACTGCGTGCCAGAAGTgRN4-FATGGAGAGTCGTGCCAGAAGTgRN4
ptg-gRNA4AGGTCTCTAAACGTCTTTAACAGTTAAACCATTGCACCAGCCGGGAATCGASP-DLGTCGTGCTCCACATGTTGACCGGSP-RCCCGACATAGATGCAATAACTTCGFaGAGACCGGTCTCGGTTTCAGAGCTATGCTGGAAACAGCGRAGCTGGAGAGGCGCGAAAAAAGCACCGACTCGGTGCCACTTTTCAAGTTGATU6-1-RGACCGGTCTCtAATCACTACTTCGTCTCTAACCATATATU6-1-RCACTGGTGCTTGTTAATCACTACTTCGTCTCTAACCATATATTFAACAAAGCACCAGTGGTCTAGTGGTAGAATAGTACCTGCCACGGTACAGACCCGGGTTRCGAGACCGGTCCCACGACGCGGAATCGAACCGGGTCTGTACCGTGGgRNA+RGAGTCTGATCGGCCACgRNA2-FGAGTCTGATAGTGATAAGATTCAGAGCgRNA4-FATGGTTTAACGATGATAAGATTCAGAGCgRNA4-FATGGTTTAACGTTAAAGACGTTTCAGAGCgRNA4-FGAGACTCGTGCCACGAGATTCAGAGCgRNA4-FATGGTTTAACGTTAAAGACGTTCAGAGCgRNA4-FTGGATACTGGTAGATATAGTTCAGAGCgRNA4-FTGGTTAACTGTTAAAGACGTTCAGAGCgRNA4-FTGGTTAACTGTTAAAGACGTTCAGAGCgRNA4-FTGGTTAACTGTTAAAGACGTTCAGAGCgRNA4-FTGAGAGATCCGTGCCAGAAGTchun0718-FTCCTCACCATGGGGTCTGCGAT
SP-DLGTCGTGCTCCACATGTTGACCGGSP-RCCGACATAGATGCAATACTTCGFaGAGACCGGTCTCGGTTCAGAGCTATGCTGGAAACAGCGRAGCTCGAGAGGCGCGAAAAAGCACCGACTCGGTGCACTTTTCAAGTTGATU6-1-RGAGACCGGTCTCAATCACTACTTCGTCTCAACCATATATU6-1-RCACTGGTGCTTGTTAATCACTACTTCGTCTCAACCATATATTFAACAAGCACCAGTGGTCAGAGCAGAATAGTAGCACGGGTCTGTAACGAGCGGGAgRNA-RCAGCGCTCTGTGCACCAGGCGGAACACGAGCCGGGACTGTACGGGTGCGACgRNA1-FCAGGTCTGTCCATCAAGATGTTCAGAGCgRNA2-FGAGTCTGGTGCATAGGTTTCAGAGCgRNA3-FATCGATGGATGATGATATAGTTCAGAGCgRNA4-FAGGTCTAACGTTAGAGCAGAGCgRNA4-FAGGTTTAACGTTAGAGCAGAGCgRNA4-FAGGATTAGGTTAGAGAGTTTCAGAGCgRNA4-FAGGATTAACGTTGCCAGAAGTgRNA4-FAGGATTAACGTGCCAGAAGTgRNA4-FAGGATTAACGTGTGCCAGAAGTgRNA4-FTGGAGATGCGTGCCAGAAGTgRNA4-FTGGAGATTCCGTGCCAGAAGTgRNA4-FGAGATTCCGTGCCAGAAGTgRNA4-FGAGATTCCGTGCCAGAAGTgRNA4-FTGGAGATTCCGTGCCAGAAGTgRNA4-FTGGAGATTCCGTGCCAGAAGTgRNA4-FTGGAGATTCCGTGCGATgRNA4-FTGGAGATTCCGTGCGATGAGAGATTCCGTGCGATGAGAGTTCCGTGCGAGAGTGAGAGATCCGGTGCGGAGAGTGAGAGATCCGGGTGCGGAGAGTGAGAGATCCGGGGTGCGGAGAGTGAGAGATCCGGGGGGGGGGGGGGGGGGGGGGGGGGGGGG
SP-RCCCGACATAGATGCAATAACTTCGFaGAGACGGGTCTCGGGTTCAGAGCAGAGAACAGCGGRAGCTCGAGAGGCGCAAAAAAGCACCGACTCGGTGCCACTTTCAAGTTGATU6-1-QGAGACCGGTCTCAATCACTACTCGTCTCAACCATATAU6-1-RCACTGGTGCTTGTAATCACTACTTCGTCTCAACCATATATTFAACAAGCACCAGTGGTCAAGAGGAGAATAGTACCCTGGCAGCAGGAGACACAGGGTTRGAGACCGGTCTCAACAGAGCGGGAATCGAACAGGGTCTGAGAGCGGGAgRNA-RCACCGACTCGGTGCCACgRNA1-FGAGCTGGTGCAAGAGGTTCAGAGCGCAGAGCGGAGACGGGAGAGAGA
GFaGAGACCGGTCTCGGTTTCAGAGCTATGCTGGAAACAGCGRAGCTCGAGAGCGCGAAAAAGCACCGACTGGTGCACTTTTCAAGTGAATU6-1-RGAGACCGGTCTCAAACACTACTCGTCTCAACCATATAU6-1-RACAGAGCACGAGTGTAATACACTACTCGTCTCAACCATATATTFAACAAGCACCAGTGGTCTAGTGGTAGAATAGTACCAGGGTACAGAGCCGGGTTRGAGACCGGTCTCGCACAGGGGAATCGAACCGGGTCTGTACCGGGAACCGGGTgRNA-RGAGCTCGCCACCAGCAGAGCGGAACCGAGAGCGGAGCGG
GRAGCTCGAGAGGCGCAAAAAGCACCGACTGGGTGCCACTTTTCAAGTTGATU6-1-QGACCGGTCAAGCAGAAATCTCAAAATCCGGCAGAACAAU6-1-QCACAGCGCTCTCAATCACTTCGTCTCAACCATATU6-1-RAACAAGCACGGTGCACACAGCGGCAATCGAACCAGGTACAGAGCGGGTTFAACAAGCACCAGTGGTGCACAGCGGAATCGAACCGGGTCTGTACCGTGGTRGAGACCGGTCCCACGAGCAGCAGCAGCGGGAATCGAACCGGGTCTGTACCGTGGgRNA-RGAGCTCTGTCCAACAGAGTGTTCAGAGCgRNA2FGAGACTGGGTGCACAGAGCGTCTGAAGAGCgRNA3FACTCGATGAGAGATATAGTTCAGAGCgRNA4FAGGTTTAACTGTTAAGACGTTTCAGAGCgRNA4FAGGTTTAACTGTTAAGACGTTTCAGAGCgRNA4FAGGATTAACTGTGCAGAAGAGTTCAGAGCgRNA4FAGGATTAACTGTGCAGAGAGTTCAGAGCgRNA4FAGGATTCAGTGTGCCAGAAGTTCAGAGCgRNA4FAGGATTCAGTGTGCCAGAAGTTCAGAGCgRNA4FAGGATTCAGTGTGCCAGAAGTTCAGAGCgRNA4FAGGATTCAGTGCCAGAAGTTCAGAGCgRNA4FAGGATTCAGTGCCAGAAGTTCAGAGCgRNA4FAGGATTCAGTGCCAGAAGTTCAGAGCgRNA4FAGGATTCAGTGCCAGAAGTTCAGAGCgRNA4FAGGAGAGCGTGCGCAGAAGTgRNA4FAGGAGAGAGTGCGCAGAGAGTgRNA4FAGGAGAGAGTGCGGGAGAGTgRNA4FAGGAGAGAGGTGCGAGAGGTgRNA4FAGGAGAGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGG
U6-1-FGACCGGTAAGGCGCGAGAAATCTCAAAATTCCGGCAGAACAAU6-1-RCGAGACCGGTCTCAACACTACTCGTCTAACCATATAU6-1-RACCTGGTGCTTGTAATCACTACTCGTCTCAACCATATATTFAACAAAGCACCAGTGGTCTAGTGGTAGAATAGTACCCTGCCACGGTACAGAGCCGGGTTRCGAGACCGGTGCCACgRNA-RGACCGACTCGGTGCCACGGAATCGAAGCGGTCTGTACCGTGGCgRNA1-FCAGGTCTGTCCAACAAGATGTTCAGAGCgRNA2-FGGAGTCGGATGATAGAGTTTCAGAGCgRNA4-FATGGTTTAACTGTTAAAGACGTTTCAGAGCgRNA4-FGAGATTCCGTGACCAGAAGTTCAGAGCch101718TCCTACTGCGTGCCAGAAGT
U6-1-CCGAGACCGGTCTCAATCACTACTCGTCTCAACCATATATU6-1-RCACTGGTGCTTGTAATCACTACTCGTCTAACCATATATTFAACAAAGCACCAGTGGTCTAGTGGTAGAATAGTACCCTGCCACGGTACAGAGCCGGGTTRCGAGACCGGTCTCGCACCACGGAATCGAACCGGGTCTGTACCGTGGgRNA-RGCACCGACTCGGTGCCACgRNA2-FGGAGTCTGATACTGGCTTAGGTTTCAGAGCgRNA3-FACTCGATGGATGATAGTTTCAGAGCgRNA4-FATGGTTTAACTGGTTGCACAGAGCgRNA4-FGGAGATCGGTGCCAGAAGTTCAGAGCAchn107181-TGAGAGATTCCGTGCCAGAAGTAchn107181-TCCTTACTGGCTGCCAGAAGT
U6-1-RCACTGGTGCTTGTTAATCACTACGTCGTAACAATAGCATATATTFAACAAAGCACGAGTGGTAGGAGAGAATAGCACGGGAACCGGGTACAGAGAGCGGGGTRCGAGACCGGTCTCTGCAACAGCGGGAATCGAACCGGGGTCTGTACGGGGGgRNA-RGCACCGACTGGGTGCCACgRNA1-FCAGGTCTGTCCCATGAGATGTTCAGAGCgRNA2-FGGAGTCTGATGATGATGATGATGAGAGCgRNA3-FACTCGATGGATGATGATGATTCAGAGCgRNA4-FACGGTTTAACGATGATGATGAGAGCgRNA4-FTGGATGATGATGATGACGAGAGAGAGAGAGAGAGAGAGAG
TFAACAAAGCACCAGTGGTCTAGTGGTAGAATAGTACCCTGCCACGGTACAGACCCGGGTTRCGAGACCGGTCTCTGCACCAGCGGGAATCGAACCCGGGTCTGTACCGTGGgRNA-RGCACCGACTGGCGCACgRNA1-FCAGGTCTGTCCCATCAAGATGTTCAGAGCgRNA2-FGGAGTCTGATGATGATGATGATGATGAGAGCgRNA3-FACTCGATGGATGATGATGATGATGAGAGCgRNA4-FATGGTTTAACTGTTAAGACGTTTCAGAGCAchn107181-FTGAGAGATCCGTTGCCCAGAAGTAchn107181-FTCCTTACCGTTGCCCAGAAGT
TRCGAGACCGGTCTCTGCACCAGCGGGAATCGAACCCGGGTCTGTACCGTGGgRNA-RGCACCGACTCGGTGCCACgRNA1-FCAGGTCTGATCACAGATGTTCAGAGCgRNA3-FGGAGTCTGATGATGATGATGATGAGAGCgRNA4-FATGGTTTAACGATGATGACGTTTCAGAGCAchn107181-FGAGAGATCCGTGCCAGAAGTAchn107181-FTCCTACGCGTGCGGAGAGCAchn107181-FTCCTACGCGGGTCGGAGAGC
gRNA-RGCACCGACTCGGTGCCACgRNA1-FCAGGTCTGTCCCATCAAGATGTTTCAGAGCgRNA2-FGGAGTCTGATACTGGCTTAGGTTTCAGAGCgRNA3-FACTCGATGGATGATGATGATAGTTTCAGAGCgRNA4-FTGGGTTTAACTGTTAAGACGTTTCAGAGCAchn107181-FTGAGAGATTCCGTTGCCCAGAAGTAchn107181-RTCCTTACTGATGGTCTGCCAGAAGT
gRNA1-FCAGGTCTGTCCCATCAAGATGTTTCAGAGCgRNA2-FGGAGTCTGATACTGGCTTAGGTTTCAGAGCgRNA3-FACTCGATGGATGATGATGATATAGTTTCAGAGCgRNA4-FATGGTTTAACTGTTAAAGACGTTTCAGAGCAchn107181-FTGAGAGATTCCGTTGCCCAGAAGTAchn107181-RTCCTTACTGATGCGTCTGCGAT
gRNA2-FGGAGTCTGATACTGGCTTAGGTTTCAGAGCgRNA3-FACTCGATGGATGATGATAGTTTCAGAGCgRNA4-FATGGTTTAACTGTTAAAGACGTTTCAGAGCAchn107181-FTGAGAGATTCCGTTGCCCAGAAGTAchn107181-RTTCCTTACTCATGCGGTCTGCGAT
gRNA3-FACTCGATGGATGATGATGATAGTTTCAGAGCgRNA4-FATGGTTTAACTGTTAAAGACGTTTCAGAGCAchn107181-FTGAGAGATTCCGTTGCCCAGAAGTAchn107181-RTTCCTTACTCATGCGGTCTGCGAT
gRNA4-FATGGTTTAACTGTTAAAGACGTTTCAGAGCAchn107181-FTGAGAGATTCCGTTGCCCAGAAGTAchn107181-RTTCCTTACTCATGCGGTCTGCGAT
Achn107181-F TGAGAGATTCCGTTGCCCAGAAGT Achn107181-R TTCCTTACTCATGCGGTCTGCGAT
Achn107181-R TTCCTTACTCATGCGGTCTGCGAT
PP2A-F GCAGCACATAATTCCACAGG
PP2A-R TTTCTGAGCCCATAACAGGAG
T1-F GTGTGGTGTTGTCTGCTCTATGTTT
T1-R CTCTCTCACATTGGTGGGGTTGT
T2-F ACAACCCCACCAATGTGAGAGAG
T2-R CCAGCGCCCAATCATTTACCCTC
T3-F AGTTGTTTGAATACTATGACCAGGATCTCCC
T3-R CATATGGCTGTCCACCTAGCATTGC
T4-F AGCAATGCTAGGTGGACAGCCATATG
T4-R GCAGCATATACGCGGAAAGAATTATAAACGAT
OT1-F TCTCCACCTTCTTTATGC
OT1-R TGCTATTTGTCCGTCTC
OT2-F ATAACCAAGGCAGAGC
OT2-R AACCCATGACCACTAAC
OT3-F TGGCAATGAACACCTC
OT3-R AACCCATGACCACTAACT
OT4-R GTTAGCGTCCCTCACA

Vector ID	sgRNA	No. of clones analyzed	No. of mutated clones	Mutation frequency (%)
A1	sgRNA1	120	9	7.50%
	sgRNA2		9	7.50%
	sgRNA1&sgRNA2		9	7.50%
A2	sgRNA3	280	0	0.00%
	sgRNA4		19	6.78%
	sgRNA3&sgRNA4		0	0.00%
B1	sgRNA1	120	109	90.83%
	sgRNA2		98	81.67%
	sgRNA1&sgRNA2		98	81.67%
B2	sgRNA3	260	190	73.07%
	sgRNA4		158	65.38%
	sgRNA3&sgRNA4		156	60.00%

Table S2. Summary of mutagenesis frequencies in positive clones selected.



Figure S1. Overall structure of vectors for CRISPR/Cas system. (a) Overall structure of the Cas9 binary expression vector for CRISPR/Cas9 system. (b) Overall structure of the Cas9 binary expression vector for PTG/Cas9 system. (c) Overall structure of the sgRNA intermediate vector for CRISPR/Cas9 system. (d) Overall structure of the sgRNA intermediate vector for PTG/Cas9 system.



Figure S2. PCR validation and Sanger sequencing of PCR products amplified from paired-sgRNAs/Cas9 vectors. (a) Validation for CRISPR/Cas9 system. (b) Validation for PTG/Cas9 system.



Figure S3. Transgenic positivity check in G418-resistance callus lines with Cas9 specific primers SP-DL/SP-R. (a) Representation of CRISPR/Cas9 system. (b) Representation of PTG/Cas9 system.



Figure S4. The relative expression of ssgRNAs. (a) The relative expression levels of sgRNA1 and sgRNA2 in kiwifruit G418-resistance callus lines transformed with A1/B1. (b) The relative expression levels sgRNA3 and sgRNA4 in kiwifruit G418-resistance callus lines transformed with A2/B2.