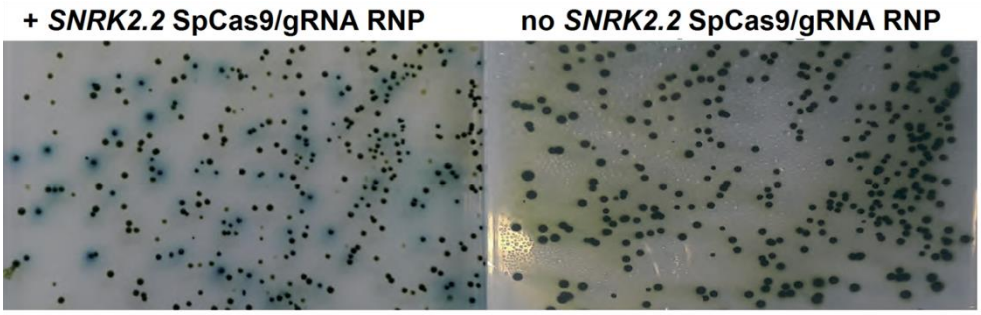
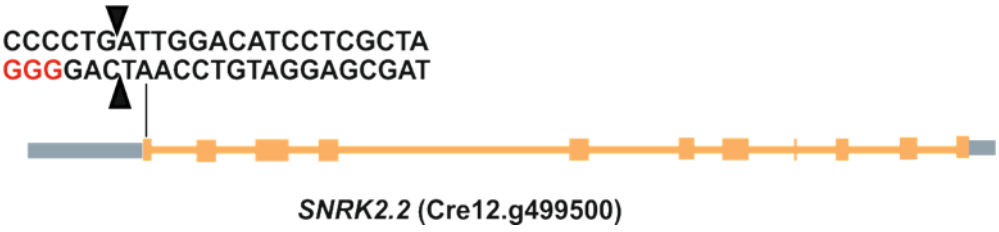
A



B



C

SNRK2.2, wild-type target

…**AGCCGGGTCCAGCAGGCCGACCAGAGC*CCC*TGAATTGGACATCCTCGCTATGAACAGGTTGGT AATTCTCAGGGTGCATAGAGGTGCTGTCCTCG**…

Donor single-stranded Oligo S-ssODN-FLAG

A*G*C*CGGGTCCAGCAGGCCGACCAGAGC*CCC*TGACTCCGTTGTAACTTTTCCTTCAATCGAA ATTGGACATCCTCGCTATGAATAGGT*T*G*G

Donor single-stranded Oligo P-ssODN-FLAG

C*C*A*ACCTaTTCATAGCGAGGATGTCCAATTTCGATTGAAGGAAAAGTTACAACGGAGTCA*G* *GG*GCTCTGGTCGGCCTGCTGGACCCG*G*C*T

Targeted insertions found in the *SNRK2.2* gene in blue colonies

1. Precise FLAG insertion

…**AGCCGGGTCCAGCAGGCCGACCAGAGC*CCC*TGACTCCGTTGTAACTTTTCCTTCAATCGAAAT TGGACATCCTCGCTATGAACAGGTTGGTAATTCTCAGGGTGCATAGAGGTGCTGTCCTCGC**…

2. The partial duplication of S-ssODN-FLAG insertion resulted in the 59 bp repeat

…AGCCGGGTCCAGCAGGCCGACCAGAGC*CCC*TGACTCCGTTGTAACTTTTCCTTCAATCGACCT
ATGCCGGGTCCAGCAGGCCGACCAGAGC*CCC*TGACTCCGTTGTAACTTTTCCTTCAATCGAAAA
TGGACATCCTCGCTATGAAAAGGTTGGTAATTCTCAGGGTGCATAGAGGTGCTGTCCTCGC…

3. The partial duplication by insertion in reverse direction of S-ssODN-FLAG resulted in the 33 bp reverse repeat

…AGCCGGGTCCAGCAGGCCGACCAGAGC*CCC*TGACTCCGTTGTAACTTTTCCTTCATCCAATTT
CGATTGAAGGAAAAGTTACAACGGAGTCAGGGGCTCTGGTGGACATCCTCGCTATGAAAAGGTT
GGTAATTCTAAGGGTGCATAGAGGTG…

4. Partial FLAG integration, accompanied by duplication of the 64 bp region adjacent to the cleavage site, resulting in the 105 bp insertion.

…GCCGGGTCCAGCAGGCCGACCAGAG*CCC*CTGATTGGACATCCTCGCTATGAAAAGGTTGGTAA
TTCTCAGGGTGCATAGAGGTGCTGTCCTCGCCCCAGCAGTCCTGGTGCTGTTGTCGCTTTTCCT
TCAATCGAAATTGGACATCCTCGCTATGAAAAGGTTGGTAATTCTCAGGGTGCATAGAGGTGCT
GTCCTCGCCC…

5. Integration of P-ssODN-FLAG accompanied by duplication of the 45 bp region adjacent to the cleavage site (nucleotide exchanges are shown in brackets) and insertion of the pPmR fragment through the 2 bp microhomology (highlighted in yellow), resulting in the 570 bp insertion.

…CAGGCCGACCAGAG*CCC*CTGATTGGACATCC(A)C(C)CTATGAAAAGG(A)TGGTAATTCTC
AGGGTGCATCCTTTTTTTCTGCCTCGCCCCTGCTGCTTGCAAACAAAAAAACCACCGCTACCAG
CGGTGGTTTGTTTGCCGGATCAAGAGCTACCAACTCTTTTTCCGAAGGTAACTGGCTTCAGCAG
AGCGCAGATACCAAATACTGTTCTTCTAGTGTAGCCGTAGTTAGGCCACCACTTCAAGAACTCT
GTAGCACCGCCTACATACCTCGCTCTGCTAATCCTGTTACCAGTGGCTGCTGCCAGTGGCGATA
AGTCGTGTCTTACCGGGTTGGACTCAAGACGATAGTTACCGGATAAGGCGCAGCGGTCGGGCTG
AACGGGGGGTTCGTGCACACAGCCCAGCTTGGAGCGAACGACCTACACCGAACTGAGATACCTA
CAGCGTGAGCTATGAGAAAGCGCCACGCTTCCCGAAGGGAGAAAGGCGGACAGGTATCCGGTAA
GCGGCAGGGTCGGAACACGAGAGCGCACGGGTCCAGCAGGCCGACCAGAGCCCCTGACTCCGTT
GTAACTTTTCCTTCAATCGAAATTGGACATCCTCGCTATGAAAAGGTTGGTAATTCTCAGGGTG
CATAGAGGTGCTGTCCCTCGCCCCA…

Figure S7 Blue-green screening and analysis of colonies containing targeted insertions in the *SNRK2.2* gene. (A) An example of screening of blue colonies containing targeted insertions in the *SNRK2.2* gene, performed directly on agar plates. Grown colonies were sprayed with 3 mM X-SO4 and blue color appeared after 24 h incubation at room temperature. CC-125 strain or mutants were transformed with *SNRK2.2* SpCas/gRNA RNP, pPmR and donor DNA. In control CC-125 strain or mutants were transformed with nonspecific SpCas/gRNA RNP, pPmR and donor DNA. (B) Schematic representation of the *SNRK2.2* gene where a recognition site of gRNA (the protospacer region) is shown above. PAM is shown in red and cleavage site is marked using triangles. (C) Examples of targeted insertions found in the *SNRK2* gene in blue colonies of the ΔKU80-C4 transformants. ΔKU80-C4 cells were transformed with *SNRK2.2* SpCas/gRNA RNP, pPmR and a single-stranded oligonucleotide template that matched either the protospacer strand (P-ssODN-FLAG) or the spacer strand (S-ssODN-FLAG) of the protospacer

region in the *SNRK2* gene. Protospacer region in the *SNRK2* gene is shown in blue, PAM is show in red italics, FLAG sequence is shown in red, and pPmR sequence is shown in violet.