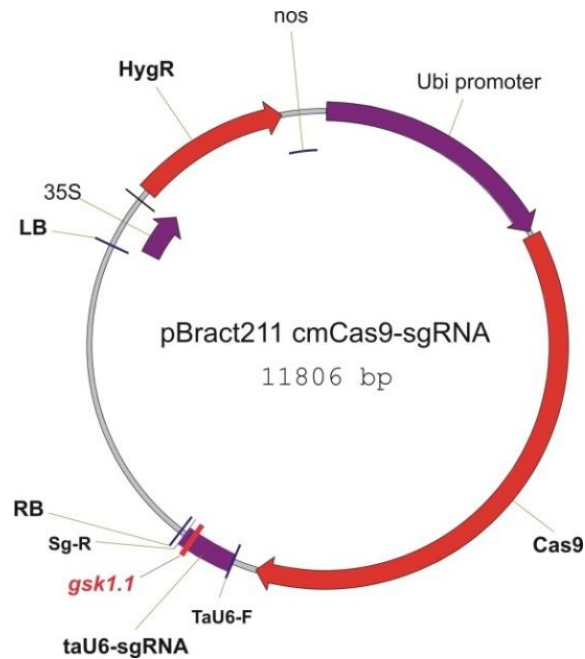


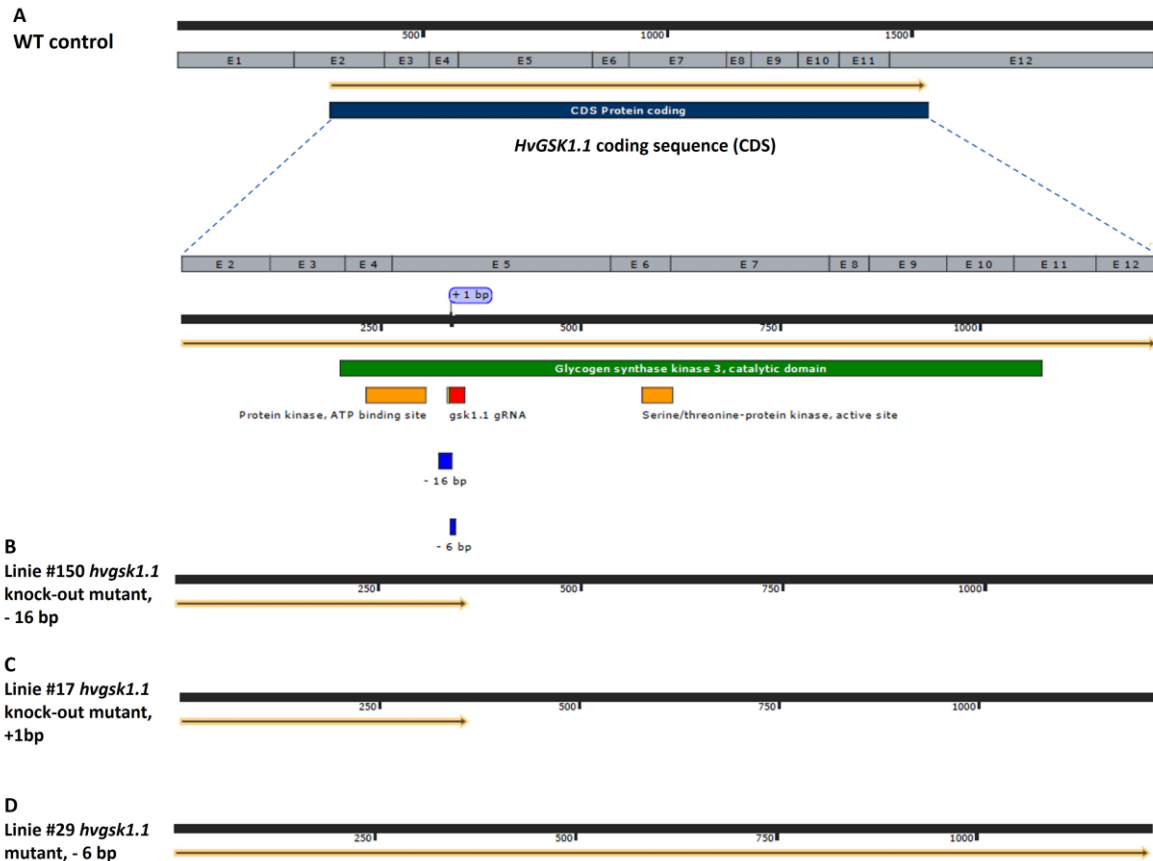
Supplementary Materials:



**Figure S1.** Scheme of the destination vector pBract211cmCas9-sgRNA. The vector pBract211cmCas9-sgRNA contains i) a synthetic Cas9 gene (based on the native sequence of *Streptococcus pyogenes* with codon usage optimized for expression in monocots) driven by the maize ubiquitin promoter; ii) an sgRNA cassette consisting of the wheat U6 RNA promoter, *gsk1.1* gRNA, and transcription termination signal, and amplification primers TaU6-F and Sg-R are indicated; iii) a hygromycin resistance gene (*hptII*) as a selection marker for the regenerated plants.

**Table S1.** List of the PCR primers and oligonucleotides that were used in this study.

Purpose	Symbol	Sequence	Oligos/Amplicon Length (bp)
oligos for cloning <i>HvGSK1.1</i> sgRNA HORVU.MOREX.r3.3HG02526 10	oligo1_gsk1_1-Fw	5'- <i>ctg</i> GCGCATGGTCTGTAGCTCA-3'	23
	oligo1_gsk1_1-Re	5'- <i>aac</i> TGAGCTACAGACCATGCGC-3'	23
PCR primers flanking the <i>hptII</i> gene	hpt-F-205	5'-GACGGCAATTTTCGATGATG-3'	205
	hpt-R-205	5'-CCGGTCGGCATCTACTCTAT-3'	
PCR primers flanking the cloning site in the pBract211_cmCas9-sgRNA-gsk1.1 vector	TaU6 – F	5'-GACCAAGCCCGTTATTCTGA-3'	375
	Oligo gsk1.1- R	5'-AAACTGAGCTACAGACCATGCGC-3'	
PCR primers flanking the TaU6sgRNA cassette	TaU6 – F	5'-GACCAAGCCCGTTATTCTGA-3'	465 – 467
	Sg – R	5'- AAAAAAAGCACCGACTCGGTGCCAC-3'	
PCR primers flanking the <i>HvGSK1.1</i> target site sequence (HORVU.MOREX.r3.3HG0252610)	gsk1.1_Ontarg_L	5'-CCTGTGGCAAACCTCCAATGC-3'	367
	gsk1.1_Ontarg_R	5'-CCTTCAGGACAAGCGCTACA-3'	
PCR primers flanking the <i>HvGSK1.1</i> target site sequence (HORVU.MOREX.r3.3HG0252610) extended by Illumina platform-specific adapters	ADAPTgsk1.1F	5'-TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGGGGTGTA CCTGGAGCTTGTG-3'	359
	ADAPTgsk1.1R	5'-GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGGCATGCGCTGGTTCATCTTG-3'	
PCR primers flanking the Off-targets HORVU2Hr1G029120	Off_ 9120F	5'-TCGTCGGCAGCGTCGATCAATGTGCTCGGCGTTG-3'	160
	Off_ 9120R	5'-GTCTCGTGGGCTCGGAACAACCTCACCTCCGGCTT-3'	



**Figure S2.** Diagram showing the structure of the *HvGSK1.1* transcript, CDS sequence, and GSK1.1 protein length WT (**A**), truncated (**B**, **C**), and with the expected deletion of two amino acids (**D**).

**A** Structure of *HvGSK1.1* transcript, E1-E12 exons, CDS (dark blue) and ORF (orange arrow), GSK3 catalytic domain (green color), protein kinase, ATP binding site, serine/threonine, protein kinase active site (orange color), gsk1.1 gRNA (red), PAM (yellow), mutations in the target site: deletion of 16 nt (-16 bp), insertion of 1 nt (+1bp), and deletion of 6 nt (-6 bp) (highlighted in blue).

**B** Predicted change in HvGSK1.1 protein length in the #150 knockout mutant line.

**C** Predicted change in HvGSK1.1 protein length in the #17 knockout mutant line.

**D** Predicted changes in HvGSK1.1 protein length in the mutant with two amino acid deletions in line #29.