

WT-A

TGATATAAACTAACTGTGGTGCATTGCTTGCCTAGATGAAGCACAGTTCAGCTATTTCCGCTGTTTTGAC
CGATGACAATTCGACAATGGCACCCCTAGAGGAAGATGTCAAGACTGAAAATATTGGCCTCCTAAATTTG
GATCCAACTTTGGAACCTTATCTAGATCACTTCAGACACAGAATGAAGAGATATGTGGATCAGAAAATGC
TCATTGAAAAATATGAGGGACCCCTGAGGAATTTGCTCAAGGTAACAGCCAAAAGTTGTGCTTTAGGCA
GTTTGACCTTATTTGGAAGATGAATTGTTTATACCTACTTTGACTTTGCTAGAGAATTTTGCATACCGG
GGAGTAAGTAGTGGCTCCATTTAGGTGGCACCTGGCCATTTTTTTGATCTTTTAAAAAGCTGTTTGATTG
GGTCTTCAAAAAAGTAGACAAGTTTTTTGGAGAAGTGACACACCCCGGAGTGTCAAGTGGCAAAGCAA

WT-B

TGATATAAACTAACTGTGGTGCATTGCTTGCCTAGATGAAGCACAGTTCAGCTATTTCCGCTGTTTTGAC
CGATGACAATTCGACAATGGCACCCCTAGAGGAAGATGTCAAGACTGAAAATATTGGCCTCCTAAATTTG
GATCCAACTTTGGAACCTTATCTAGATCACTTCAGACACAGAATGAAGAGATATGTGGATCAGAAAATGC
TCATTGAAAAATATGAGGGACCCCTGAGGAATTTGCTCAAGGTAACAGCCAAAAGTTGTGCTTTAGGCA
GTTTGACCTTATTTGGAAGATGAATTGTTTATACCTACTTTGACTTTGCTAGAGAATTTTGCATACCGG
GGAGTAAGTAGTGGCTCCATTTAGGTGGCACCTGGCCATTTTTTTGATCTTTTAAAAAGCTGTTTGATTG
GGTCTTCAAAAAAGTAGACAAGTTTTTTGGAGAAGTGACACACCCCGGAGTGTCAAGTGGCAAAGCAA

WT-C

TGATATAAACTAACTGTGGTGCATTGCTTGCCTAGATGAAGCACAGTTCAGCTATTTCCGCTGTTTTGAC
CGATGACAATTCGACAATGGCACCCCTAGAGGAAGATGTCAAGACTGAAAATATTGACCTCCTAAATTTG
GATCCAGCTTTGGAACCTTATCTAGATCACTTCAGATACAGAATGAAGAGATATGTGGATCAGAAAATGC
TCATTGAAAAATATGAGGGACCCCTGAGGAATTTGCTCAAGGTAACAGCCAAAAGTTGTGCTTTAGGCA
GTTTGACCTTATTTGGAAGATGAATTGTTTATACCTACTTTAACTTTGCTAGAGAATTTTTCATACCGG
GGAGTAAGTAGTGGCTCCATTTAGGTGGCACCTGGCCATTTTTTTGATCTTTTAAAAAGCTGTTTGATTG
GGTCTTCAAAAAAGTAGACAAGTTTTTTGGAGAAGTGACACACCCCGGAGTGTCAAGTGGCAAAGCAA

Supplementary Figure S1. Nucleotide sequences of the region around the target sites in the WT-A, WT-B, and WT-C genomes. PCR primer sites used for sequence analysis are highlighted by yellow-colored letters. Polymorphic nucleotides between WT-A and others are highlighted in green, and those between WT-C and others are highlighted in blue. *Bam*HI sites are shown as red letters. Region for the predicted exon 3 is boxed. Target sites of the gRNAs are underlined.

#30-C (-165)

TGATATAAACTAACTGTGGTGCATTGCTTGCCTAGATGAAGCACAGTTCAGCTATTTCCGCTGTTTTGAC
CGATGACAATTCGACAATGGCACCCCTA----- 165 nt -----
-----AAGTTGTGCTTTAGGCA
GTTTGACCTTATAATTGGAAGATGAATTGTTTATACCTACTTTAACTTTGCTAGAGAATTTTCATACCGG
GGAGTAAGTAGTGGCTCCATTTAGGTGGCACCTGGCCATTTTTTTCGATCTTTTAAAAAGCTGTTTGATTG
GGTCTTCAAAAAAGTAGACAAGTTTTTTGGAGAAGTGACACACCCCGGAGTGTCTAGTGGCAAAGCAA

#31-B (-138)

TGATATAAACTAACTGTGGTGCATTGCTTGCCTAGATGAAGCACAGTTCAGCTATTTCCGCTGTTTTGAC
CGATGACAATTCGACAATGGCACCCCTTGAGGAA----- 138 nt -----
-----TTTGTCTCAAGGTAACAGCCAAAAGTTGTGCTTTAGGCA
GTTTGACCTTATAATTGGAAGATGAATTGTTTATACCTACTTTGACTTTGCTAGAGAATTTTCATACCGG
GGAGTAAGTAGTGGCTCCATTTAGGTGGCACCTGGCCATTTTTTTCGATCTTTTAAAAAGCTGTTTGATTG
GGTCTTCAAAAAAGTAGACAAGTTTTTTGGAGAAGTGACACACCCCGGAGTGTCTAGTGGCAAAGCAA

#86-B (-138)

TGATATAAACTAACTGTGGTGCATTGCTTGCCTAGATGAAGCACAGTTCAGCTATTTCCGCTGTTTTGAC
CGATGACAATTCGACAATGGCACCCCT----- 138 nt -----
TGAGGAATTTGTCTCAAGGTAACAGCCAAAAGTTGTGCTTTAGGCAGTTTGACCTTATAATTGGAAGATGAA
TTGTTTATACCTACTTTGACTTTGCTAGAGAATTTTCATACCGGGGAGTAAGTAGTGGCTCCATTTAGG
TGGCACCTGGCCATTTTTTTCGATCTTTTAAAAAGCTGTTTGATTGGGTCTTCAAAAAAGTAGACAAGTT
TTTTGGAGAAGTGACACACCCCGGAGTGTCTAGTGGCAAAGCAA

#86-C (-165)

TGATATAAACTAACTGTGGTGCATTGCTTGCCTAGATGAAGCACAGTTCAGCTATTTCCGCTGTTTTGAC
CGATGACAATTCGACAATGGCACCCCTA----- 165 nt -----
-----AAGTTGTGCTTTAGGCA
GTTTGACCTTATAATTGGAAGATGAATTGTTTATACCTACTTTAACTTTGCTAGAGAATTTTCATACCGG
GGAGTAAGTAGTGGCTCCATTTAGGTGGCACCTGGCCATTTTTTTCGATCTTTTAAAAAGCTGTTTGATTG
GGTCTTCAAAAAAGTAGACAAGTTTTTTGGAGAAGTGACACACCCCGGAGTGTCTAGTGGCAAAGCAA

#99-C (-223, +59)

TGATATAAACTAACTGTGGTGCATTGCTTGCCTAGATGAAGCACAGTTCAGCTATTTCCGCTGTTTTGA-
----- 223 nt -----TAATTTGTACGTAGCAT
AGATACATAGACACAAAATGTCCTCCCATTGAGCTAGCCG(59 nt insertion)-----
-----ATTGGAAGATGAATTGTTTATACCTACTTTAACTTTGCTAGAAAATTTTCATACCGG
GGAGTAAGTAGGGGCTCCATTTAGGTGGCACCTGGCCATTTTTTTCGATCTTTTAAAAAGCTGTTTGATTG
GGTCTTCAAAAAAGTAGACAAGTTTTTTGGAGAAGTGACACACCCCGGAGTGTCTAGTGGCAAAGCAA

#115-C (-86)

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TGATATAAACTAACTGTGGTGCATTGCTTGCCTAGATGAAGCACAGTTCAGCTATTTCCGCTGTTTTGAC
CGATGACAATTCGACAATGGCACCCCTAGAGGAAGATGTCAAGACTGAAAAT----- 86 nt ----GC
TCATTGAAAAATATGAGGGACCCCTGAGGAATTTGCTCAAGGTAACAGCCAAAAGTTGTGCTTTAGGCA
GTTTGACCTTATAATTGGAAGATGAATTGTTTATACCTACTTTAACTTTGCTAGAGAATTTTTCATACCGG
GGAGTAAGTAGTGGCTCCATTTAGGTGGCACCTGGCCATTTTTTTCGATCTTTTAAAAAGCTGTTTGATTG
GGTCTTCAAAAAAGTAGACAAGTTTTTTGGAGAAGTGACACACCCCGGAGTGTTCAGTGGCAAAGCAA
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#115-C (-108)

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TGATATAAACTAACTGTGGTGCATTGCTTGCCTAGATGAAGCACAGTTCAGCTATTTCCGCTGTTTTGAC
CGATGACAATTCGACAATGG----- 108 nt -----ATCAGAAAATGC
TCATTGAAAAATATGAGGGACCCCTGAGGAATTTGCTCAAGGTAACAGCCAAAAGTTGTGCTTTAGGCA
GTTTGACCTTATAATTGGAAGATGAATTGTTTATACCTACTTTAACTTTGCTAGAGAATTTTTCATACCGG
GGAGTAAGTAGTGGCTCCATTTAGGTGGCACCTGGCCATTTTTTTCGATCTTTTAAAAAGCTGTTTGATTG
GGTCTTCAAAAAAGTAGACAAGTTTTTTGGAGAAGTGACACACCCCGGAGTGTTCAGTGGCAAAGCAA
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Supplementary Figure S2. Nucleotide sequences of the mutant genes. PCR primer sites used for sequence analysis are highlighted in yellow. Region for the predicted exon 3 is boxed. Gaps indicate the deletions in the gene. Inserted sequences are shown as blue letters. Polymorphic nucleotides are highlighted in blue and green.

Potato SBE3	MEINFNVLSPKPIRGSPSPSPKVVSSGASRNKICFP SQHSTGLKFGSQERS	50
Rice BE1	MLCLTSSSSSAPAPLLPSLA-----DRPSPGIAGGGGNV	34
Potato SBE3	WDISSSTPKSRVRKDERMKHSSAISAVLTDNSTMABLEEDVKTENIGLLN	100
Rice BE1	-RLSVVSSPRRSWP GKVKTNF SVPA TARKNKTMVTVV EE -VDHLPI--YD	80
Potato SBE3	LDPTLEPYLDHFRHRMKRYVDQKMLIEKYEGPLEEFAQGYLKFGFNRE DG	150
Rice BE1	LDPKLEEFKDFNFYRIKRYLDQKCLIEKHEGGLEEF SKGYLKFGINTVDG	130
Potato SBE3	CIVYREWAPAAQEA EAVIGDFNGWNGSNHMM EKDFGVV SIRIPD VDSKPV	200
Rice BE1	ATIYREWAPAAQEAQLIGEFNNWNGAKHKMEKDKFGIWSIKISHVNGKPA	180
Potato SBE3	IPHNSRVKFRFKHGNVWVDRIPAWIKYATADATKFAAPYDGVYWDPPPS	250
Rice BE1	IPHNSKVKFRFRHGGGAWVDRIPAWIRYATFDASKFGAPYDGVHWDPPAC	230
Potato SBE3	ERYHFKYPRPPKPRAPRIYEAHVGMSSSEPRVNSYREFADDVLPRIKANN	300
Rice BE1	ERYVFKHPRPPKPDAPRIYEAHVGMSGEEPEVSTYREFADNVLPRIRANN	280
Potato SBE3	YNTVQLMAIMEHSYYGSFGYHVTNFFAVSNRYGNPEDLKYLIDKAHSLGL	350
Rice BE1	YNTVQLMAIMEHSYYASFGYHVTNFFAVSSRS GTPEDLKYLVDKAHSLGL	330
Potato SBE3	QVLVDVVHSHASNNTDGLNGFDIGQGSQESYFHAGERGYHKLWDSRFLN	400
Rice BE1	RVLMDVVHSHASNNTDGLNGYDVGNTHESYFHTGDRGYHKLWDSRFLN	380
Potato SBE3	YANWEVLRFLLSNLRWVLEEYNFDFGRFDGITSMLYVHHGINMGFTGNYN	450
Rice BE1	YANWEVLRFLLSNLRWVMEDEFMDFGRFDGVTSM LYHHHGINKGFTGN YK	430
Potato SBE3	EYFSEATDVDAVVYLM LANLIHKIFPDATVIAEDVSGMPGLSRPVSEGG	500
Rice BE1	EYFSLDTDVDAIVYMLLANHLMHKLLPEATIVAEDVSGMPVLCRPVDEGG	480
Potato SBE3	IGFDYRLAMAIPDKWIDYLNKNKDEDWSMKEVTSSLTNRRYTEKCIAYAE	550
Rice BE1	VGFDFRLAMAIPDRWIDYLNKNKEDRKWSMSEIVQTLTNRRYTEKCIAYAE	530
Potato SBE3	SHDQSI VGDKTIAFL LMDKEMYSGMSCLTDASPVVDRGIALHKMIHFFTM	600
Rice BE1	SHDQSI VGDKTIAFL LMDKEMYTGMSDLQPASPTINRGIALQKMIHFITM	580
Potato SBE3	ALGGEGYLNFMGNEFGHP EWIDFPREGNNWSYDKCRRQWNLADSEHLRYK	650
Rice BE1	ALGGDGYLNFMGNEFGHP EWIDFPREGNNWSYDKCRRQW SLVDTDH LRYK	630
Potato SBE3	FMNAFDRAMNSLDEKFSFLASGKQIVSSMDDD NKVVVFERGDLV FVFNFH	700
Rice BE1	YMNAFDQAMNALEEEFSFLSSSKQIVSDMNEKDKVIVFERGDLV FVFNFH	680
Potato SBE3	PNNTYEGYKVGCDLPGKYRVALDSDAWEFGGHGRAGHDVDHFTSPEGIPG	750
Rice BE1	PNKTYKGYKVGCDLPGKYRVALDSDALVFGGHGRVGHVDHFTSPEGMPG	730
Potato SBE3	VPETNFNGRPN SFKVLSPARTCVAYYRVDERMSE TEDYQTDICSELLPTA	800
Rice BE1	VPETNFNNRPN SFKVLSPRPTCVAYYRVDE DREELRRGGAVASGKIVTEY	780
Potato SBE3	NIEESDEKLDSSSTNISSTSKNAYYRVDERMSEAE DYQTDICSELLPT	850
Rice BE1	IDVEATSGETISGGWKSEKDDCGKKGKMFVFRSSEEDCK* 820	
Potato SBE3	ANIEESDEKLDSSLSTNISNIGQTVVVSVEERDKELKDSPSVSIISDAVP	900
Potato SBE3	AEWADSDANVWGED* 914	

Supplementary Figure. S3. Comparison of potato SBE3 and rice BE1. The deduced amino acid sequences of potato SBE3 (PGSC0003DMT400009981) and rice BE1 (BAF20543) were aligned. Identical amino acid residues are highlighted. Gaps are introduced to maximize the homology. Predicted transit peptides are indicated by red letters.