

Table S1. The primers used in study.

Prime name	Sequence (5'-3')	Gene/vector	Ref.
Actin-F	TGATGGTGTCTAGCCACACT	<i>OsActin</i>	[16]
Actin-R	TGGTCTTGGCAGTCTCCATT		
HSBP1-F	TGTTTTGCTTTGGAGGTAGCA	<i>OsHSBP1</i>	
HSBP1-R	TGTAATGCAACGACAACCCT		
HPT-F	AAACTGTGATGGACGACACCGT	<i>HPT</i>	[16]
HPT-R	GTGGCGATCCTGCAAGCTCC		
Ubi-F	CCCTGCCTTCATACGCTATT	pCas9	[16]
Cas9-t-R	GCCTCGGCTGTCTCGCCA		
BtgZI-gRNA1-F	TGTT[GACAAAACCTCCTAACCCAGA]	gRNA1	
BtgZI-gRNA1-R	AAAC[TCTGGGTTAGGAGGTTTTGTC]		
BsaI-gRNA2-F	GTGT[GCACTGCACTAGATGAAAT]	gRNA2	
BsaI-gRNA2-R	AAAC[ATTTTCATCTAGTGCAGTGC]		
RT-HSBP1-F	TCAATCCTCACCCGGGAAATCG	<i>OsHSBP1</i>	[10]
RT-HSBP1-R	CACAATGTTCTGGGACATAG		
RT-HSP70.3-F	TGAGGGAGGCTGAGCTACAT	<i>OsHSP70.3</i>	[28]
RT-HSP70.3-R	CAACTTCCTATTACATGAAC		
RT-HSP80.2-F	CGACGACGAGCAGTATGT	<i>OsHSP80.2</i>	[26]
RT-HSP80.2-R	CCAGATGTTCCTCCCACT		
RT-HSP90.2-F	TCATCACCTCCACTCTCTGC	<i>OsHSP90.2</i>	[25]
RT-HSP90.2-R	ATCAGCTTTGGACTCCCAGA		

Table S2. Segregation analysis of T-DNA components and identification of homozygous mutants in the T₁ generation.

T ₀ Plant	Total T ₁ plants screened	T-DNA free	Homozygous mutant
Hs1-134	60	15	2
Hs1-138	62	15	2
Hs1-159	58	14	1
Hs1-200	58	1	0
Total	238	45	5

crRNA1-OsHSBP1										
WT	TTTTACCGAT	TATATCTCTC	TTTCAGGTAC	AAACCTCCT	AACCCA	GAT	GGTGAGTTCC	TTCGGATATG		wt
Hs1-7-a1	TTTTACCGAT	TATATCTCTC	TTTCAGGTAC	AAACCTCCT	AACCC	T	GGTGAGTTCC	TTCGGATATG		-AGA
Hs1-7-a2	TTTTACCGAT	TATATCTCTC	TTTCAGGTAC	AAACCTCCT	AACCCA	GAT	GGTGAGTTCC	TTCGGATATG		wt
Hs1-10-a1	TTTTACCGAT	TATATCTCTC	TTTCAGGTAC	AAACCT			GGTGAGTTCC	TTCGGATATG		-12
Hs1-10-a2	TTTTACCGAT	TATATCTCTC	TTTCAGGTAC	AAACCTCCT	AACC	AGAT	GGTGAGTTCC	TTCGGATATG		-C
Hs1-14-a1	TTTTACCGAT	TATATCTCTC	TTTCAGGTAC	AAACCTCCT	AA	GAT	GGTGAGTTCC	TTCGGATATG		-CCCA
Hs1-14-a2	TTTTACCGAT	TATATCTCTC	TTTCAGGTAC	AAACCTCCT	AACCCA	GAT	GGTGAGTTCC	TTCGGATATG		wt
Hs1-43-a1	TTTTACCGAT	TATATCTCTC	TTTCAGGTAC	AAACCTCCT	AACCCAGAT		GGTGAGTTCC	TTCGGATATG		+C
Hs1-43-a2	TTTTACCGAT	TATATCTCTC	TTTCAGGTAC	AAACCTCCT	AACCTAGAT		GGTGAGTTCC	TTCGGATATG		+T
Hs1-44-a1	TTTTACCGAT	TATATCTCTC	TTTCAGGTAC	AAACCTCCT	AA	CAGAT	GGTGAGTTCC	TTCGGATATG		-CC
Hs1-44-a2	TTTTACCGAT	TATATCTCTC	TTTCAGGTAC	AAACCTCCT	AACC	CAGAT	GGTGAGTTCC	TTCGGATATG		wt
Hs1-74-a1	TTTTACCGAT	TATATCTCTC	TTTCAGGTAC	AAACC			TTCC	TTCGGATATG		-19
Hs1-74-a2	TTTTACCGAT	TATATCTCTC	TTTCAGGTAC	AAACCTCCT	AACCCA	GAT	GGTGAGTTCC	TTCGGATATG		wt
Hs1-75-a1	TTTTACCGAT	TATATCTCTC	TTTCAGGTAC	AAACCTCCT	AACCCA	T	GGTGAGTTCC	TTCGGATATG		-AG
Hs1-75-a2	TTTTACCGAT	TATATCTCTC	TTTCAGGTAC	AAACCTCCT	AACCCA	GAT	GGTGAGTTCC	TTCGGATATG		wt
Hs1-83-a1	TTTTACCGAT	TATATCTCTC	TTTCAGGTAC	AAACCTCCT	AACCCAAGAT		GGTGAGTTCC	TTCGGATATG		+A
Hs1-83-a2	TTTTACCGAT	TATATCTCTC	TTTCAGGTAC	AAACCTCCT	AACCCA	GAT	GGTGAGTTCC	TTCGGATATG		wt
Hs1-101-a1	TTTTACCGAT	TATATCTCTC	TTTCAGGTAC	AAACCTCCT	AACC	GAT	GGTGAGTTCC	TTCGGATATG		-CA
Hs1-101-a2	TTTTACCGAT	TATATCTCTC	TTTCAGGTAC	AAACCTCCT	AA	CAGAT	GGTGAGTTCC	TTCGGATATG		-CC
Hs1-129-a1	TTTTACCGAT	TATATCTCTC	TTTCAGGTAC	AAACCTCCT	AA	GAT	GGTGAGTTCC	TTCGGATATG		-ACCC
Hs1-129-a2	TTTTACCGAT	TATATCTCTC	TTTCAGGTAC	AAACCTCCT	AACC	CAGAT	GGTGAGTTCC	TTCGGATATG		wt
Hs1-134-a1	TTTTACCGAT	TATATCTCTC	TTTCAGGTAC	AAACCTCCT	AACC	AGAT	GGTGAGTTCC	TTCGGATATG		-C
Hs1-134-a2	TTTTACCGAT	TATATCTCTC	TTTCAGGTAC	AAACCTCCT	AACC	AGAT	GGTGAGTTCC	TTCGGATATG		-C
Hs1-138-a1	TTTTACCGAT	TATATCTCTC	TTTCAGGTAC	AAACCTCCT	AACC	AGAT	GGTGAGTTCC	TTCGGATATG		-C
Hs1-138-a2	TTTTACCGAT	TATATCTCTC	TTTCAGGTAC	AAACCTCCT	AACC	AGAT	GGTGAGTTCC	TTCGGATATG		-C
Hs1-157-a1	TTTTACCGAT	TATATCTCTC	TTTCAGGTAC	AAACCTCCT	AACCCAGAT		GGTGAGTTCC	TTCGGATATG		+C
Hs1-157-a2	TTTTACCGAT	TATATCTCTC	TTTCAGGTAC	AAACCTCCT	AA	CAGAT	GGTGAGTTCC	TTCGGATATG		-CC
Hs1-159-a1	TTTTACCGAT	TATATCTCTC	TTTCAGGTAC	AAACCTCCT	AACCCAAGAT		GGTGAGTTCC	TTCGGATATG		+A
Hs1-159-a2	TTTTACCGAT	TATATCTCTC	TTTCAGGTAC	AAACCTCCT	AACCCAGAT		GGTGAGTTCC	TTCGGATATG		+G
Hs1-175-a1	TTTTACCGAT	TATATCTCTC	TTTCAGGTAC	AAACCTCCT	AACCCAGAT		GGTGAGTTCC	TTCGGATATG		+C
Hs1-175-a2	TTTTACCGAT	TATATCTCTC	TTTCAGGTAC	AAACCTCCT	AACCTTAGAT		GCTTGAATAT	GTTCATATG		Sub
Hs1-193-a1	TTTTACCGAT	TATATCTCTC	TTTCAGGT				GGTGAGTTCC	TTCGGATATG		-21
Hs1-193-a2	TTTTACCGAT	TATATCTCTC	TTTCAGGTAC	AAACCTCCT	A	T	GGTGAGTTCC	TTCGGATATG		-7
Hs1-200-a1	TTTTACCGAT	TATATCTCTC	TTTCAGGTAC	AAACCTCCT	AAC	AT	GGTGAGTTCC	TTCGGATATG		-CCAG
Hs1-200-a2	TTTTACCGAT	TATATCTCTC	TTTCAGGTAC	AAACCTCCT	AACCCA	GAT	GGTGAGTTCC	TTCGGATATG		wt
Hs1-202-a1	TTTTACCGAT	TATATCTCTC	TTTCAGGTAC	AAACCTCCT	AA	CAGAT	GGTGAGTTCC	TTCGGATATG		-CC
Hs1-202-a2	TTTTACCGAT	TATATCTCTC	TTTCAGGTAC	AAACCTCCT	AACCCA	GAT	GGTGAGTTCC	TTCGGATATG		wt

Figure S1. Sanger sequencing analysis of the gRNA1 target region in representative T₀ mutant plants.

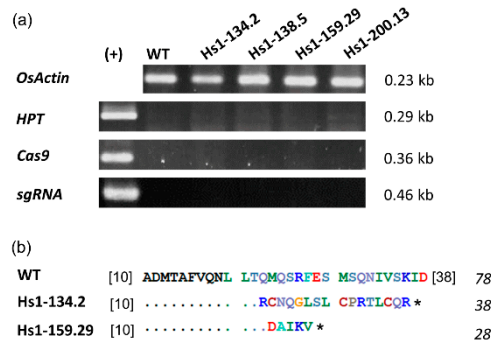


Figure S2. Identification of transgene-free homozygous *OsHSBP1* mutant lines in the T₁ generation. (a) PCR screening of T₁ plants for T-DNA elimination using primers specific for *Cas9*, *HPT*, sgRNA, and internal control *OsActin*. (+): plasmid positive control; WT: wild-type BT7. (b) Predicted amino acid sequence alignment of wild-type *OsHSBP1* and homozygous mutant lines. Deletions at the gRNA1 target site resulted in frameshift mutations and premature stop codons (marked with asterisks), leading to truncated proteins. The mutation size (in amino acid residue) is indicated on the right.