Supplementary Material



Figure S1. Time-course analysis of seed germination of *osbzip09* mutants (*osbzip09-3* and *osbzip09-4*) under normal conditions.



Figure S2. Schematic diagram of the CRISPR/Cas9 target site in Osb*ZIP09* and the basis of the mutation in *osbzip09-2*. The protospacer adjacent motif (PAM) is highlighted in blue.

k off target					
# CRISPR-GE targetDesign MM	EJ-KO primerDesign	offTarget	seqDownload	Help	

Results for job ID: 202101291132593386132593454113259338613259865711325933861325934541132593386132599656nxFmb PAM type: "NGG"

Genome: Oryza sativa japonica (Nipponbare, IRGSP1.0)

ID Target sequence		Length	GC (%)	Potential on/off-target sites (Max score) ⑦		Pairing with sgRNA (>=8 nt) (2)		
	1	GTACAGCCTCA	CCCTCGACG AGG	23 bp	65.0	[1.000; 0.304; 0.036] (<u>cl</u>	ose)	None
	Off-targ	get predictor						
	Chrom	Position	Sequence		Off-sco	re Gene	Region	
	chr01	34568188	GTACAGCCTCACCCT	CGACG AGG	1	OS01G0813100	cds Osb	zip09
	chr03	18456444	GTTCAGCCCCACCCT	ATCG AGG	0.304		intergeni	0
	chr08	21552264	GTACTGCGTCAGCAT	CGACG AGG	0.036	OS08G0442300	CDS	
	chr03	23781919	GTCGTGCCTCAGCCT	CGACG AGG	0.035	OS03G0625201	CDS	
	chr09	8824411	GAACAGCCTCTCCCT	CCGCG AGG	0.025	For off target test	intergeni	c
	chr01	4864591	GAACAGCCTCTCCCT	CGCG AGG	0.025	For on target test	intergeni	c
	chr03	13823659	GAACAGCCTCTCCCT	CCGCG AGG	0.025		intergeni	c
	chr04	2794900	GAACAGCCTCTCCCT	CGCG AGG	0.025		intergeni	c
	chr03	13873364	GAACAGCCTCTCCCT	CCCCG AGG	0.025		intergeni	c
	chr08	8697149	GAACAGCCTCTCCCT	CCCCG AGG	0.025		intergeni	c
	ohr04	9470804	GGACAGCCTCTCCCT	CGCG AGG	0.024		intergeni	c
	chr01	21652628	GGACAGCCTCTCCCT	CGCG AGG	0.024		intergeni	c
	chr08	20299012	GGACAGCCTCTCCCT	CGCG AGG	0.024		intergeni	c
	chr08	15250622	GGACAGCCTCTCCCT	CGCG AGG	0.024		intergeni	c
	chr01	35292241	GGACAGCCTCTCCCT	CGCG AGG	0.024		intergeni	- c
	chr07	19218896	GGACAGCCTGTCCCT	CACG AGG	0.024		intergeni	c
	chr04	26063228	GGACAGCCTGTCCCT	CACS AGG	0.024		intergeni	- -
	chr07	29145405	GTACAGCCTGACGCT	ALCS AGG	0.008	050760686100	CDS	
	chr02	886097	GTACTGCCTCCCCCT	GCCG ACG	0.003	050260116700	CDS	
	obr05	20683922	GTTCAGCCGCACCCT		0.002	080560421750	CDS	
Os	08g044	2300	Ref sea:		PAM CCTCGTCG	ATGCTGACGCAGT	٩C	
		С	osbzip09-2: AT o					
Os(03g062	5201	Ref seq:	200	PAM CCTCGTCG	AGGCTGAGGCACG.	AC	
		05	sbzip09-2: A G C	ссббсб МММ				

Figure S3. Off target analysis of *osbzip09-2* mutant. (A) Off target analysis of *OsbZIP09* target sequence for CRISPR/Cas9 mediated gene editing using CRISPR-GE (http://skl.scau.edu.cn/home/). (B) Sequencing result of two candidate genes with the highest off-scores indicated that there was no off target mutation in *osbzip09-2* mutant.



OsbZIP09 ...NRESAARSRARKQAYTNELENKVSRLEEENVRL...

Figure S4. Schematic representation of the bZIP domain and the corresponding amino-acid sequences in OsbZIP09. The sterotypical bZIP domain consists of a basic DNA-binding region (green) and an adjacent ZIP domain (yellow).



Figure S5. Quality analyses of the RNA-seq data. (A) Preprocessing results of sequencing data quality in wild-type ZH11 treated with ABA or mock-treated, and *osbzip09* treated with mock (n = 3). (B) Mapping result of RNA reads of RNA-seq data. (C) Analysis of the read distribution in each sample.



Figure S6. Venn diagram showing the overlap between genes responsive to ABA and mutation of *OsbZIP09*.

Primer Names	Sequence $(5' \rightarrow 3')$	Usage			
OsbZIP09-SG-seq	GTACAGCCTCACCCTCGACG	SG sequence for editing OsbZIP09			
OsbZIP09seq-F	ATCTCGTCTCGATCATGGGG	Primers for sequencing and			
OsbZIP09seq-R	CCCCAGCTTTGACCAGGAAA	confirming mutation of OsbZIP09			
bZIP09qRT-F	GAGGCATGTCAGGGGATGTG	For qRT-PCR analysis of OsbZIP09			
bZIP09qRT-R	ACTTGGGTTCTGGTACAGGC				
LEA3qRT-F	TCACTTCAAATTCGGTGCAA	For qRT-PCR analysis of LEA3			
LEA3qRT-R	CACACCCGTCAGAAATCCTC				
LEA4qRT-F	CAGCACAGGCTCCATAAGCA	For qRT-PCR analysis of LEA4			
LEA4qRT-R	CAGCACAGAGAGACATGCGT				
LEA18qRT-F	TCCCATCCATGTCCAGGTTGA	For qRT-PCR analysis of LEA18			
LEA18qRT-R	ACGGCCTCGGATGATGAAGC				
LEA25qRT-F	AGCTGGTGGACTAGGAACCG	For qRT-PCR analysis of LEA25			
LEA25qRT-R	ATGGTGTCGGGTGTTGTGG				
PP2C51RT-F	TGACGAGTTGGAACGAGTGG	For qRT-PCR analysis of PP2C51			
PP2C51RT-R	CTCGCCAGGATCAGGAACTC				
USPqRT-F	GTCCGTGGTGCTGAAGATGT	For qRT-PCR analysis of USP			
USPqRT-R	GCGACTACTTTGCCTTGACG				
LOX2qRT-F	AAGAACGCCCTCGACATCAA	For qRT-PCR analysis of LOX2			
LOX2qRT-R	CGAACTTGGACTCTCCCGTC				
CSLA5qRT-F	CACCGCCCGGAGAAACAA	For qRT-PCR analysis of CSLA5			

Table S1 Oligonucleotide primer sequences used in this study

CSLA5qRT-R	CACCACCAGCATCACCGACAT			
Actin1qRT-F	CCAAGGCCAATCGTGAGAAGA	Reference gene for qRT-PCR assay		
Actin1qRT-R	AATCAGTGAGATCACGCCCAG			
pZIP09-EcoRI-F	CGGAATTCATGGCGTCGAAGGCCGGAG	For construction of OsbZIP09-62-SK		
pZIP09-KpnI-R	GGGGTACCTCAGAAATCTGCGGAGCTTG			
proLEA25KpnI-F	GGGGTACCTCCTTGCTTTTCTCGTTTGA	For	construction	of
proLEA25BamH-R	CGGGATCCTGTGGTGGGTGAAGCGAGGA	LEA25pro-pGreenII		
proLOX2KpnI-F	GGGGTACCAGCACGTTTTTCAACGCCC	For	construction	of
proLOX2NcoI-R	CATGCCATGGTCTCTCACTAACCAACCAATGCT	LOX2pro-pGreenII		
DAPseq Adapter	CACGACGCTCTTCCGATCT	For DAP so	a compling	
А		TOI DAI -Se	eq sampning	
DAPseq Adapter	GATCGGAAGAGCACACGTCTG			
В				