Plant Biotechnology Journal

Supporting Information

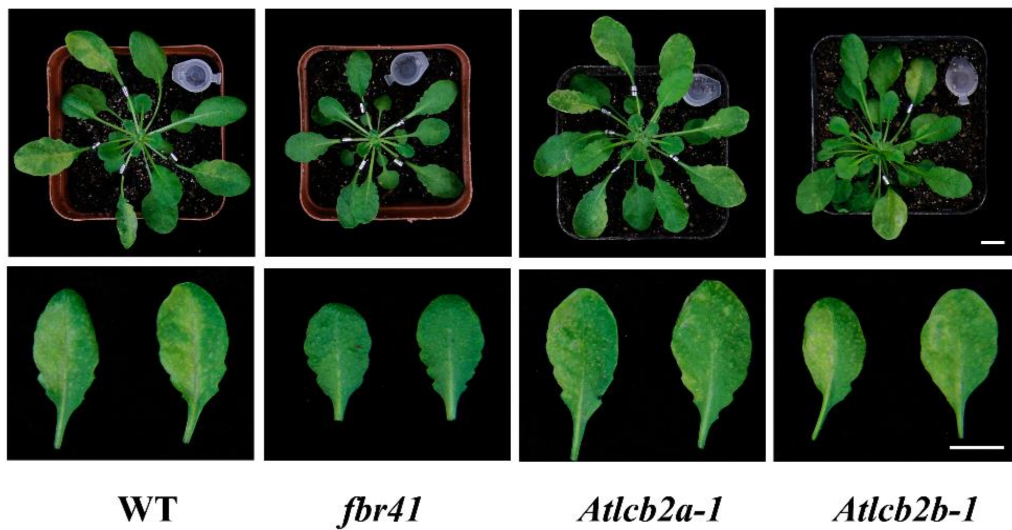

Figure S1 Lesion formation upon fumonisin B1 (FB1) injection in *fbr41* and null mutants for *AtLCB2a* and *AtLCB2b*.

Six-week-old plants were infiltrated with 10 μM FB1 (left side of treated leaves) and 1 % (v/v) methanol as control (right side of treated leaves), then photographed at 3 days post-injection (DPI). Scale bar = 1 cm.

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FBR41      MITIPYLTAVSTYFSYGLLFAFGQLRDYSRLIFDWWRTNNLQILDTLRSAWRMRISTSDD   60
AtLCB2b    MITIPYLTAVSTYFSYGLLFAFGQLRDYSRLIFDWWRTNNLQGYAPICLAHEDFYIRRLY   60
AtLCB2a    MITIPYLTAVSTYFSYGLLFAFGQLRDFFRRFIDWWFTSNLQGYAPICLGHEDFYIRRLY   60

FBR41      CIIGFRTVLDVPFQVHLMLGLMWLRESLTITTRR--------------------------   94
AtLCB2b    HRIQDCFGRPISSAPDAWIDVVERVSDDNNKTLKRTTKTSRCLNLGSYNYLGFGSFDEYC  120
AtLCB2a    HRIQDCFERPISSAPDAWFDVVERYSNDNNKTLKRTTKTSRCLNLGSYNYLGFGSFDEYC  120

FBR41      ------------------------------------------------------------
AtLCB2b    TPRVIESLKKFSASTCSSRVDAGTTSVHAELEDCVAKYVGQPAAVIFGMGYATNSAIIPV  180
AtLCB2a    TPRVIESLKKFSASTCSSRVDAGTTSVHAELEECVTRFVGKPAAVVFGMGYATNSAIIPV  180

FBR41      ------------------------------------------------------------
AtLCB2b    LIGKGGLIISDSLNHTSIVNGARGSGATIRVFQHNTPGHLEKVLKEQIAEGQPRTHRPWK  240
AtLCB2a    LIGKGGLIISDSLNHSSIVNGARGSGATIRVFQHNTPSHLERVLREQIAEGQPRTHRPWK  240

FBR41      ------------------------------------------------------------
AtLCB2b    KIIVVVEGIYSMEGEICHLPEIVSICKKYKAYVYLDEAHSIGAIGKTGRGVCELLGVDTS  300
AtLCB2a    KIIVVVEGIYSMEGEICHLPEVVAICKKYKAYVYLDEAHSIGAIGKTGKGICELLGVDTA  300

FBR41      ----------*-------------------------------------------------
AtLCB2b    DVDIMMGTFTKSFGSCGGYIAGSKDLIQYLKHQCPAHLYATSISTPSATQIISAIKVILG  360
AtLCB2a    DVDVMMGTFTKSFGSCGGYIAGSKELIQYLKHQCPAHLYATSIPTPSAQQIISAIKVILG  360

FBR41      ------------------------------------------------------------
AtLCB2b    EDGSNRGAQKLARIRENSNFFRAELQKMGFEVLGDNDSPVMPIMLYNPAKIPAFSRECLR  420
AtLCB2a    EDGSNRGAQKLARIRENSNFFRAELQKMGFEVLGDNDSPVMPIMLYNPAKIPAFSRECLR  420

FBR41      ------------------------------------------------------------
AtLCB2b    ENLAVVVVGFPATPLLLARARICISASHSREDLIKALQVISKAGDLTGIKYFPAAPKKQE  480
AtLCB2a    QKVAVVVVGFPATPLLLARARICISASHSREDLIRALKVISKVGDLSGIKYFPAEPKKIE  480

FBR41      ---------
AtLCB2b    VEKNGIKLD                                                    489
AtLCB2a    QSKNDIKLD                                                    489
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Figure S2 Comparison of amino acid sequences of FBR41, AtLCB2b and AtLCB2a. *FBR41* encodes a putative protein with the first 42 amino acids of the annotated 489 amino acids of AtLCB2b plus 52 additional amino acids before premature stop codon. The region in grey background indicates the identical amino acid sequence of FBR41 and AtLCB2b. AtLCB2b and AtLCB2a have conserved pyridoxal 5'-phosphate-binding site (GTFTKSFG) (marked with boxes), and pyridoxal 5'-phosphate-binding lysine residue (marked with asterisk), which are essential for SPT function.

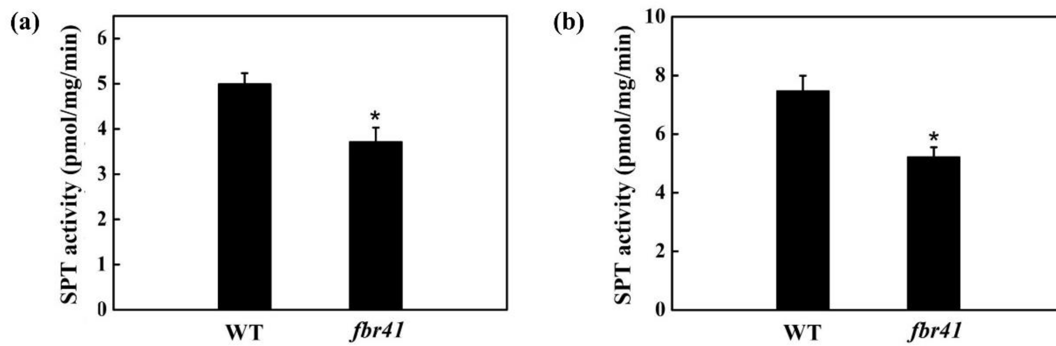


Figure S3 *In planta* SPT activity in wild-type (WT) and *fbr41* mutant

(a-b) SPT activity of microsomes prepared from untreated (a) or FB1-treated (b) leaves of WT and *fbr41* mutant. In (a), rosette leaves were harvested directly from six-week-old plants for microsomes preparation. In (b), rosette leaves were injected with 10 μM FB1 and harvested 6 h after toxin treatment for microsomal isolation. Data shown are means ± SD of three biological replicates. Asterisks denote statistically significant differences compared with WT (Student's *t* test, $^*P < 0.05$)

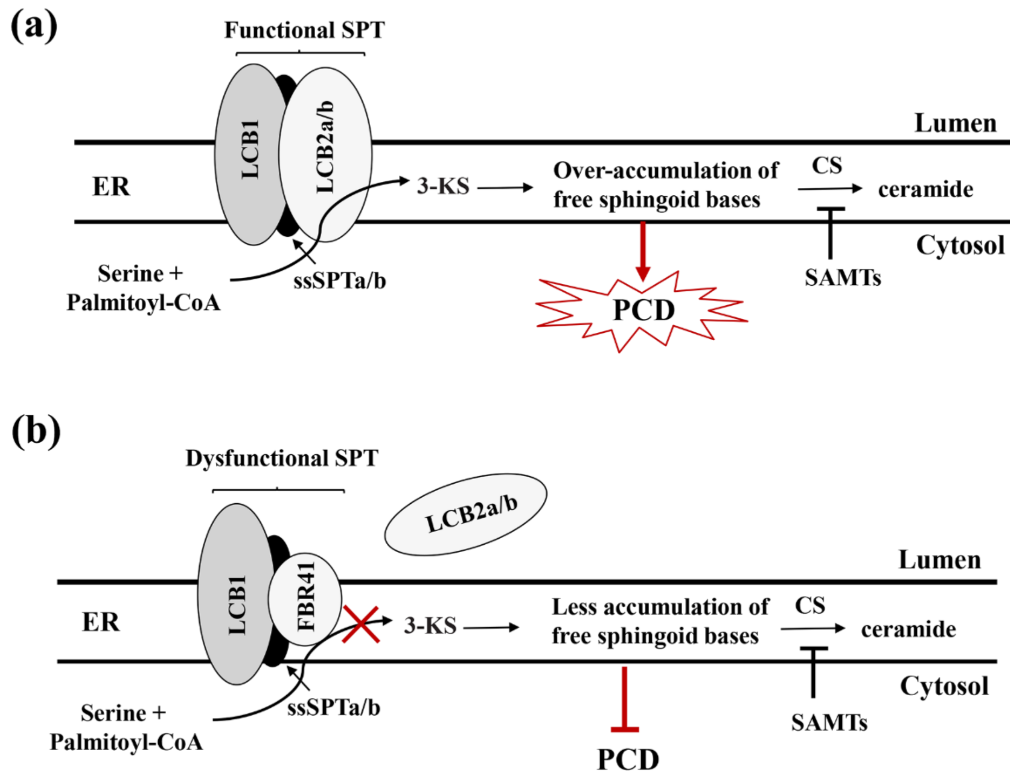


Figure S4 Proposed model considering the role of FBR41 in the resistance to sphinganine analog mycotoxin (SAMT)-induced cell death.

(a) Upon SAMT stress, free sphingoid bases are over-accumulated, leading to the induction of programmed cell death (PCD). (b) FBR41 competes with functional LCB2 subunit for LCB1 binding, yielding a dysfunctional serine palmitoyltransferase (SPT), thereby attenuating the accumulation of free sphingoid bases and inhibiting the induction of PCD. ER, endoplasmic reticulum; 3-KS, 3-ketosphinganine; CS, ceramide synthase.

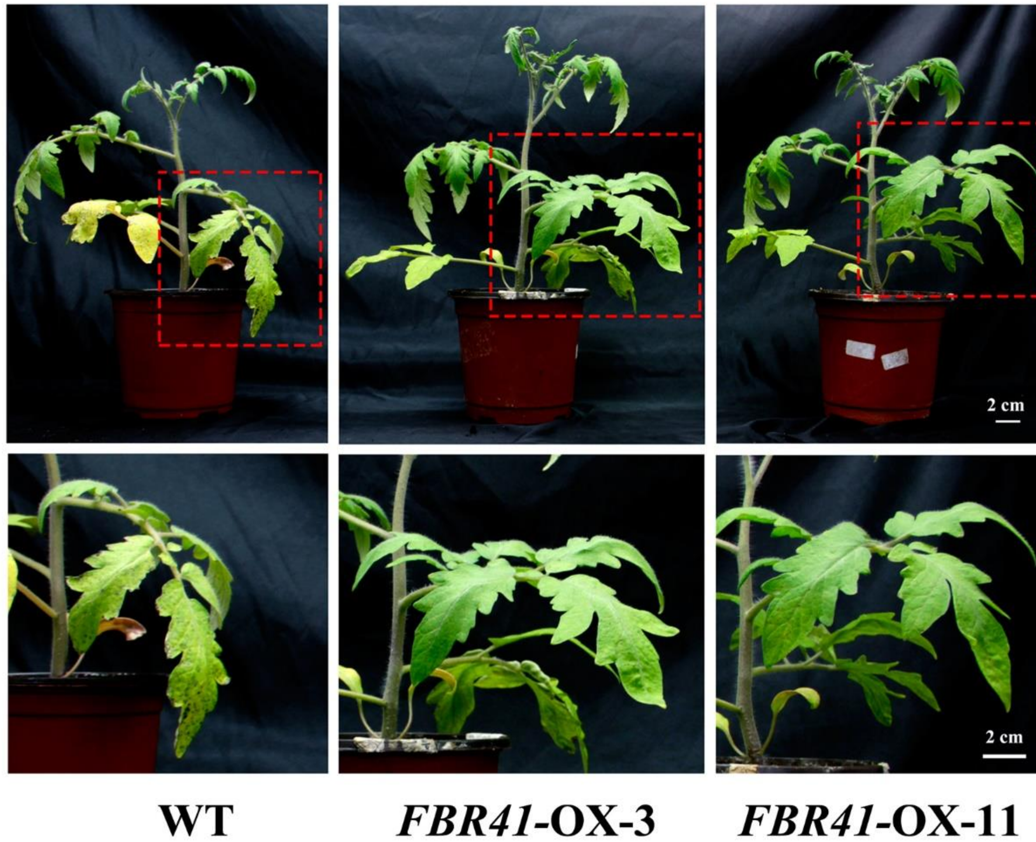
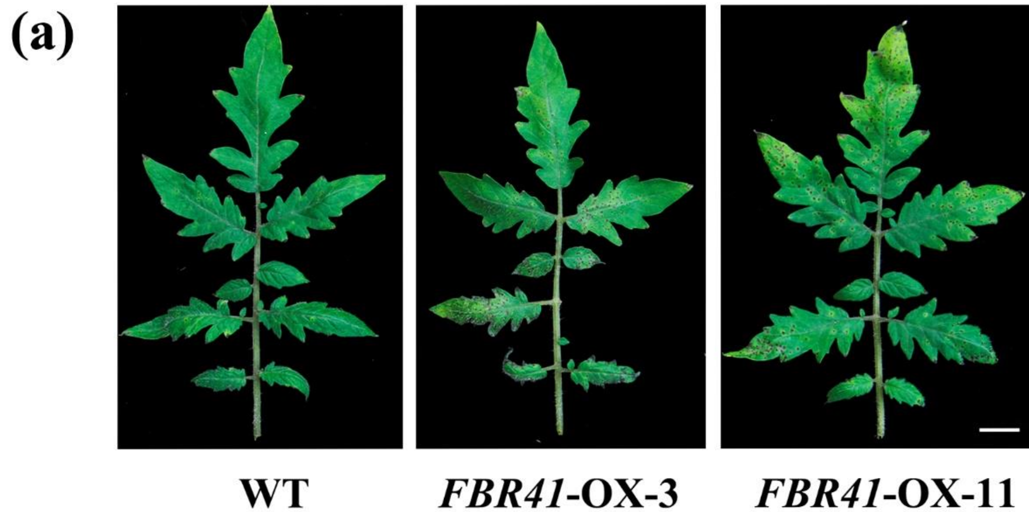


Figure S5 The disease symptoms of wild-type (WT) and *FBR41*-overexpressing transgenic plants after AAL inoculation.

Five-week-old plants were inoculated with AAL and disease symptoms were photographed at 3 days post inoculation (DPI). Top panel shows the disease symptom of plants and the dashed boxes indicate the representative foliar symptoms. The bottom panel shows the magnified images of foliar symptoms.



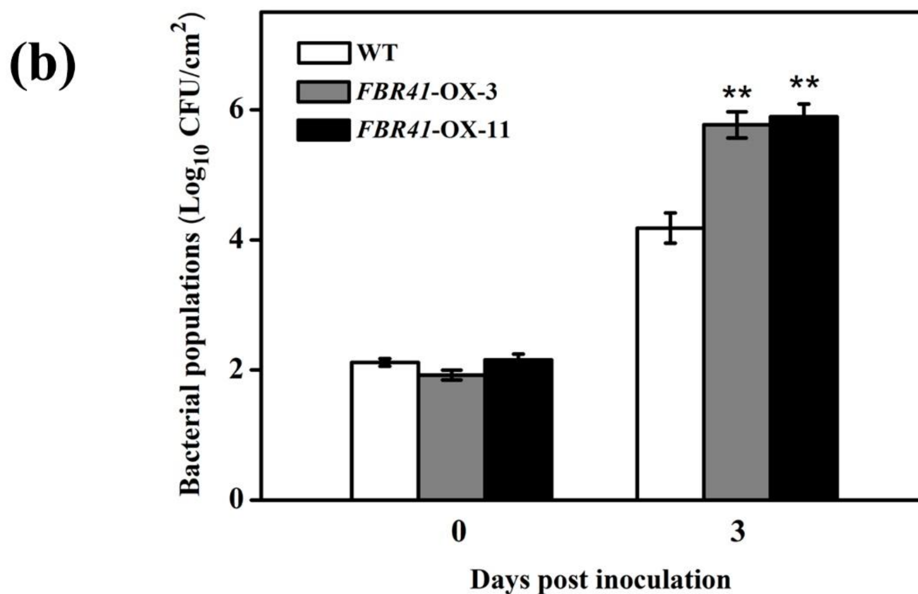


Figure S6 *FBR41*-overexpressing tomato exhibited decreased resistance against *Pseudomonas syringae* pv. tomato DC 3000 (*Pst* DC3000).

(a) Foliar disease symptom of wild-type (WT) and *FBR41*-overexpressing tomato plants after *Pst* DC3000 infection. Five-week-old plants were inoculated with *Pst* DC3000 and pictures were taken at 3 days post inoculation (DPI). Scale bar =1 cm.

(b) Bacterial population of *Pst* DC3000 in WT and transgenic plants on the day of inoculation (0 DPI) and 3 DPI. Data shown are means ± SD of three biological replicates. Asterisks denote statistically significant differences from WT(Student's *t* test, $**P < 0.01$).

Table S1 Fumonisin B1-resistant mutants identified by forward genetic screening in *Arabidopsis*

Mutant	Location of mutation site	Phenotypes	Mutagenesis	Dominant/Recessive	Reference
fbr1	Lower arm of chromosome I	FB1-resistant, no morphological alterations	EMS-mutagenesis	Recessive	(Stone *et al*., 2000)
fbr2	Upper arm of chromosome V	FB1-resistant, no morphological alterations	EMS-mutagenesis	Recessive	(Stone *et al*., 2000)
fbr6	*SQUAMOSA promoter binding protein-like 14* (*At1g20980*)	FB1-resistant, enhanced leaf margin serration and elongated petioles	T-DNA insertion	Recessive	(Stone *et al*., 2005)
fbr11-1	*Long chain base 1* (*At4g36480*)	FB1-resistant, no morphological alterations	T-DNA insertion	Recessive	(Shi *et al*., 2007)
fbr12	*Eukaryotic translation initiation factor 5A-2* (*At1g26630*)	FB1-resistant, antiapoptotic, reduced dark-induced leaf senescence, severe defects in plant growth and development	T-DNA insertion	Recessive	(Feng *et al*., 2007)
fbr136	Chromosome III	FB1-resistant, no morphological alterations	EMS-mutagenesis	Recessive	(Sun & Zuo, 2008)

EMS: ethyl methanesulfonate

Table S2 List of SSLP markers used for first-pass mapping

Maker	Chromosome	Forward primer (5’-3’)	Reserve primer (5’-3’)
NGA 63	Chr1	AACCAAGGCACAGAAGCG	ACCCAAGTGATCGCCACC
NGA392	Chr1	TTGAATAATTTGTAGCCATG	GGTGTTAAATGCGGTGTTC
NGA392	Chr1	GTCGTCTATGATGGGAAATAC	ATGCTAAAAGGGTCATAAGGGC
NGA280	Chr1	CTGATCTCACGGACAATAGTGC	GGCTCCATAAAAAGTGCACC
T8K14	Chr1	GGACAAGAACCTCATACCTTGC	CCCCATTCACCCACTCTTGACTC
T12J2	Chr2	TGAACCCTTATAATATGGCTGGC	GGTAAGCAAGGAAAGGAACAATTC
NGA1126	Chr2	GGTAAGCAAGGAAAGGAACAATTC	CGCTACGCTTTTCGGTAAAG
NGA168	Chr2	TCGTCTACTGCACTGCCG	GAGGACATGTATAGGAGCCTCG
T30B22	Chr2	TGGCCATTCACTTGATCGCCATG	CTGACATCCAGTTTGGTAACTAC
NGA162	Chr3	CATGCAATTTGCATCTGAGG	CTCTGTCACTCTTTTCCTCTGG
MMJ24	Chr3	AAAACAGGAAGCAAGAAAAATGAG	TCGAACATAAGTGAGAAATAGATG
T24C20	Chr3	GCACACATTTGTTATGTTAT	TAGCCGAACCGCACCTTT
T18N16	Chr3	GAGTAAAGTTTGCGTTGTAT	GTTCTTCCGAGTTGATTCTA
NGA6	Chr3	TGGATTTCTTCCTCTCTTCAC	ATGGAGAAGCTTACACTGATC
T15B16	Chr4	CAAATTTCACTGATTCATCGC	TGATTGTTCGATTTTGTAGTTG
F24G24	Chr4	AAAACTGACTTAATGGGTTAGCC	GAAGTAGAGGGAACAATCGGATGC
F1N20	Chr4	ATATGGTAGGCATTGTTGACATG	CACATCATAGTTAACTTGCGTTTC
NGA1107	Chr4	GCGAAAAAACAAAAAAATCCA	CGACGAATCGACAGAATTAGG
NGA225	Chr5	GAAATCCAAATCCCAGAGAGG	TCTCCCCACTAGTTTTGTGTCC
NGA106	Chr5	GTTATGGAGTTTCTAGGGCACG	TGCCCCATTTTGTTCTTCTC
NGA76	Chr5	GGAGAAAATGTCACTCTCCACC	AGGCATGGGAGACATTTACG
K3K3IND	Chr5	TGGTTCAGATCATGATCAAGTAG	CGACGAGTGATGGGGTTACAAAAG
MXC20	Chr5	CAGTGTAGCAGAAAAGGATTAAC	GGGTTAAGAGAAATGATAGAGATG

Table S3 List of CAPS markers used for fine mapping

Marker	Chromosome	Forward primer (5’-3’)	Reverse primer (5’-3’)
CAPS18005	Chr3	GATTCGGTCTGAACACAAAG	GTCTTGGTATGCTTCCATGC
CAPS18066	Chr3	ATCAGTAATCACATAAGTGG	TGCAAAGTCCTTCAATGGC
CAPS18170	Chr3	GGAATAGAGCATAAGCAGTTAAGAG	GTGTGGATACAACGTCCCGGAACGAC
CAPS18175	Chr3	GCCAAAGTATCTACTTAAGAAAGC	GTAGCAATTGACATACCCAATC
CAPS18254	Chr3	ATCTCAGATACCAAGCCAGA	GCAAGGATCTTCACCAACAT
CAPS18711	Chr3	CACATGCGCTTAGC	CTTGCCATAAGGAC

Table S4 Primers used in this study

Primer name	sequence (5'-3')	Experiments
48780-genomic-F	CTTCTCGCGTACCGCAAAACGCAG	Amplifying At3g48780 genomic DNA
48780-genomic-R	GTCAGGAAACAAAAGGGAAGAGTTTG	
48780-cDNA-F	AATACACATAACATTCCCTAATAAG	Amplifying At3g48780 cDNA
48780-cDNA-R	CGAAAAAGATTTTAATCCCTTTATA	
FBR41-CDS-F	ATGATTACGATCCCATACCTTA	Generating 35S::FBR41-myc
FBR41-CDS-R	ACGTCTTGTTGTTATCGTCAGA	
AtLCB1-Nluc (Kpn I)-F	GGggtaccATGGCTTCGAATCTCGTG	Generating AtLCB1-Nluc
AtLCB1-Nluc (Sal I)-F	ACGCgtcgacGGACTTGAGTAGAAGCTCTGA	
AtLCB1-Cluc-(Kpn I)-F	GGggtaccATGGCTTCGAATCTCGTG	Generating AtLCB1-Cluc
AtLCB1-Cluc-(Sal I)-R	ACGCgtcgacTCAGGACTTGAGTAGAAGCTCT	
AtLCB2a-Nluc-(Kpn I)-F	GGggtaccATGATAACGATTCCTTATTTAACC	Generating AtLCB2a-Nluc
AtLCB2a-Nluc-(Sal I)-R	ACGCgtcgacATCCAGCTTGATGTCGTTTTTC	
FBR41-Cluc-(Kpn I)-F	GGggtaccATGATTACGATCCCATACCT	Generating FBR41-Cluc
FBR41-Cluc-(Sal I)-R	ACGCgtcgacTTAACGTCTTGTTGTTATCG	
FBR41-F	ATGATTACGATCCCA	Identification of transgenic plants
MYC-R	CACCGTTCAAATCTTCTTC	
At3g48780-qPCR-F	TTTGCGAGAAAACTTGGCGG	Gene expression analysis
At3g48780-qPCR-R	CCGGTAAGGTCACCTGCTTT	
FBR41-qPCR-F	TGCTTTTCGCGTTTGGTCAG	Gene expression analysis
FBR41-qPCR-R	CCAAGCAGATCGGAGCGTAT	
AtACTIN7-qPCR-F	CCATTCAGGCCGTTCTTTC	Internal control
AtACTIN7-qPCR-R	CGTTCTGCGGTAGTGGTGA	
SlACTIN7-qPCR-F	TGGTCGGAATGGGACAGAAG	Internal control
SlACTIN7-qPCR-R	CTCAGTCAGGAGAACAGGGT	
DeH-F	CTCCGCCTGCCAATGTGATTAC	Estimation of AAL biomass
E8T7	GCGTACCAAGGCACGTGCTCAA	

Methods S1 Screening of the fumonisin B1-resistant mutant.

M2 seeds from ethyl methanesulfonate (EMS)-generated mutant population were sown on half-strength MS medium containing 1% (w/v) sucrose and 0.7% (w/v) agar with 1 μM FB1 for 21 d. The seedlings which can survive were transferred to half-strength MS medium without FB1 and allowed to self-fertilized to produce M3 seeds. M3 seeds were subjected to 1 μM FB1 to confirm FB1-resistant phenotype. Prior to phenotypic analysis, the mutant was backcrossed to wild-type Col-0 three times.

Methods S2 tomato transformation

Cotyledons excised from tomato seedlings (7-10 days old, grown under sterile condition) were incubated for 15 min in a suspension of *Agrobacterium tumefacien* LBA4404 in MS salts containing 3 % sucrose. The infected cotyledons were transferred to shoot-inducing medium containing MS, 3 % sucrose (w/v), 0.8 % (w/v) agar, 1 mg L^{-1} IAA and 1.75 mg L^{-1} zeatin. After 2 days co-cultivation, explants were transferred to the same medium supplemented with 75 mg L^{-1} kanamycin and 200 mg L^{-1} timentin and cultivated until the production of callus. Explants were subcultured onto shoot-inducing medium with 50 mg L^{-1} kanamycin and 200 mg L^{-1} timentin. Finally, induced shoots were rooted on root-inducing medium containing MS, 3 % sucrose (w/v), 0.8 % (w/v) agar, 50 mg L^{-1} kanamycin and 200 mg L^{-1} timentin. Shoots that rooted in the presence of kanamycin were transferred to soil.

Methods S3 *Pseudomonas syringae* pv tomato DC3000 infection assays

Inoculation of *Pst* DC3000 was carried out as previously described (Li *et al*., 2014). In brief, bacterial was cultured overnight in king's B liquid medium containing 25 μg/ml rifampicin, and then collected and resuspended in infiltration buffer (10 mM $MgCl_2$, 0.04 % Silwet L–77) to OD_{600} = 0.0002. Five-week-old tomato plants were vacuum infiltrated with bacterial suspension or infiltration buffer (Mock) and kept in the growth chamber with high humidity. Disease symptoms were observed daily, and the *in planta* growth of bacterial was measured according to Li *et al.* (2014).

Methods S4 Microsome preparation and SPT assay

Isolation of microsomes from *Arabidopsis* leaves were performed as previously described (Chen *et al*., 2006, Lynch and Fairfield, 1993) and the protein concentration were determined using Bradford method (Beyotime, Haimen, China) following the manufacturer's protocols. SPT assay was performed as previously described (Han *et al.*, 2002).

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