Plant Communications, Volume *5*

Supplemental information

The root-knot nematode effector Mi2G02 hijacks a host plant trihelix transcription factor to promote nematode parasitism

Jianlong Zhao, Kaiwei Huang, Rui Liu, Yuqing Lai, Pierre Abad, Bruno Favery, Heng Jian, Jian Ling, Yan Li, Yuhong Yang, Bingyan Xie, Michaël Quentin, and Zhenchuan Mao

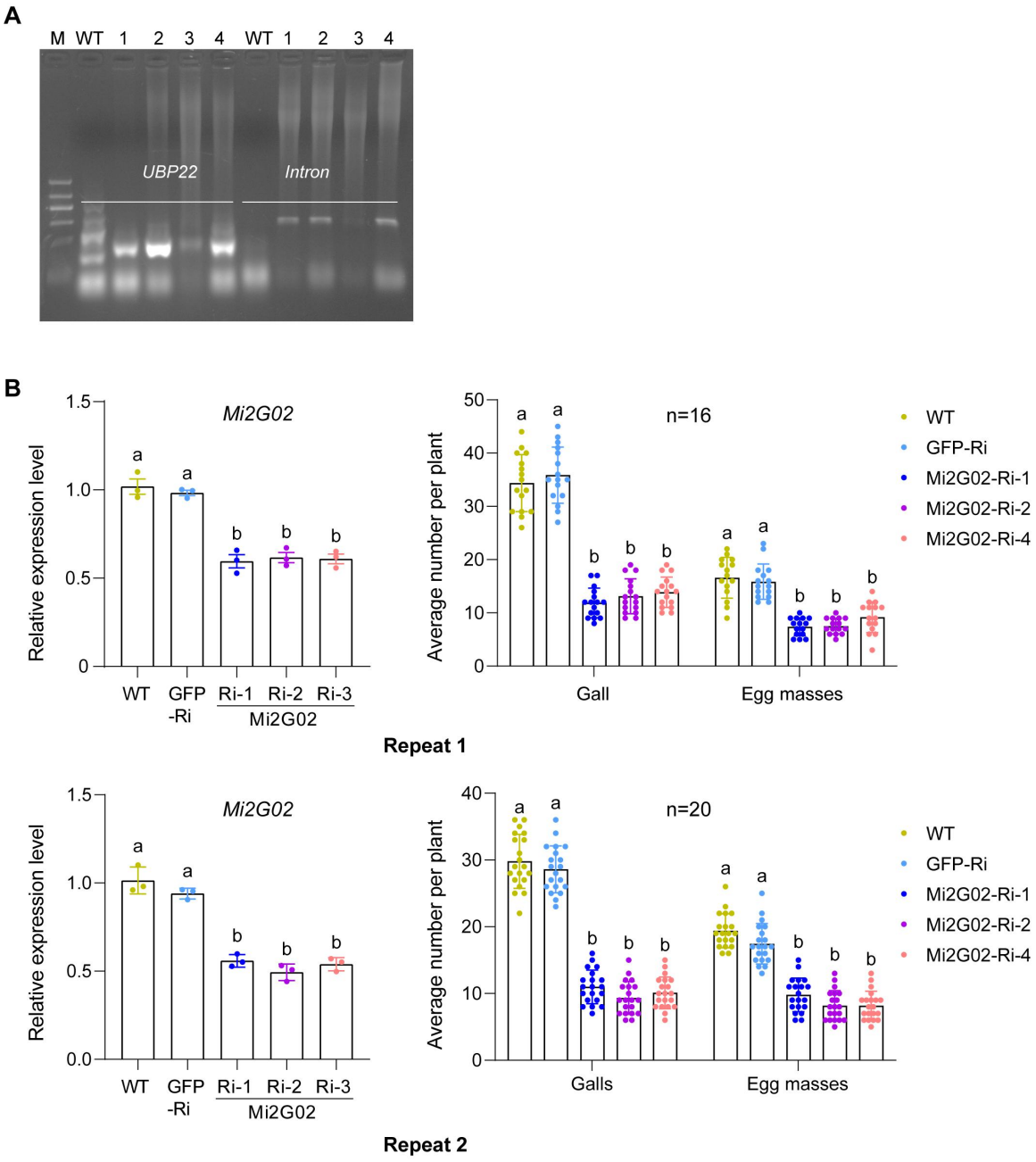
Supplemental Information

A root-knot nematode effector Mi2G02 hijacks a host plant trihelix transcription factor for nematode parasitism

Jianlong Zhao[1,4,*], Kaiwei Huang[1,4], Rui Liu[1,4], Yuqing Lai[1], Pierre Abad[2], Bruno Favery[2], Heng Jian[3], Jian Ling[1], Yan Li[1], Yuhong Yang[1], Bingyan Xie[1], Michaël Quentin[2,*] and Zhenchuan Mao[1,*]

[1] State Key Laboratory of Vegetable Biobreeding, Institute of Vegetables and Flowers, Chinese Academy of Agricultural Sciences, 100081, Beijing, China
[2] INRAE, Université Côte d'Azur, CNRS, ISA, F-06903, Sophia Antipolis, France
[3] Department of Plant Pathology and Key Laboratory of Pest Monitoring and Green Management of the Ministry of Agriculture, China Agricultural University, 100193, Beijing, China
[4] These authors contributed equally: Jianlong Zhao, Kaiwei Huang, Rui Liu.

*** Corresponding:** Email: Jianlong Zhao (zhaojianlong@caas.cn), Michaël Quentin (michael.quentin@inrae.fr), Zhenchuan Mao (maozhenchuan@caas.cn).

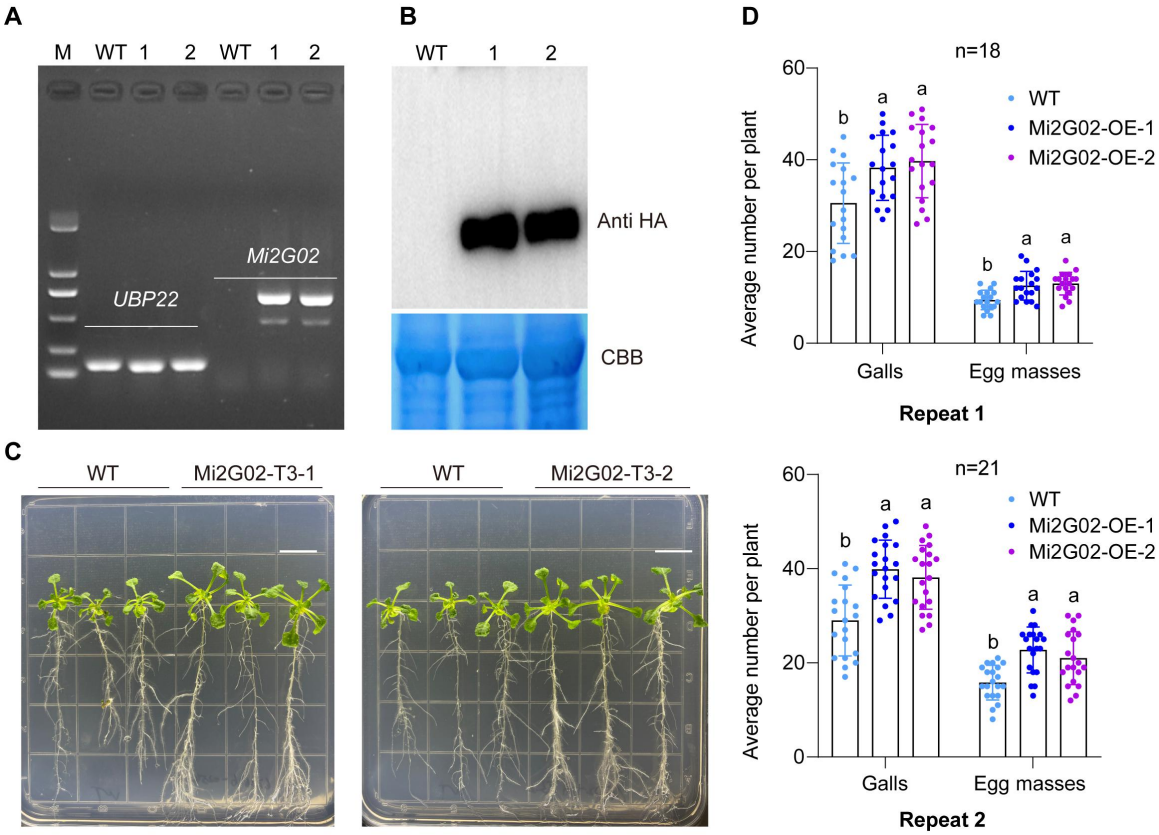


Supplemental Figure 1. Verification of the homozygous *Mi2G02* RNAi *Arabidopsis thaliana* lines and analysis of their susceptibility to *Meloidogyne incognita*.

(A) PCR was performed to verify intron expression using genomic DNA from wild-type (WT) and four independent *Mi2G02* RNAi *A. thaliana* lines (1, Mi2G02-

Ri-1; 2, Mi2G02-Ri-2; 3, Mi2G02-Ri-3; 4, Mi2G02-Ri-4). *UBP22* (*AT5G10790*) was used as an internal control.

(B) Two independent nematode infection assays using *Mi2G02* RNAi *A. thaliana* lines. *Mi2G02* expression level in nematodes recovered from three homozygous RNAi lines (Mi2G02-Ri-1, Mi2G02-Ri-2 and Mi2G02-Ri-4), gfp-RNAi line (GFP-Ri) and wild-type (WT) were determined at 10 days post-infection dpi) with *M. incognita* by RT-qPCR. *GAPDH* was used as an internal control. The values shown are means ± SE (n = 3). Gall numbers and egg masses were counted at 35 dpi. Values are presented as means ± SD (n=16 or n=20). Different letters indicate significant differences (P<0.05, one-way ANOVA).



Supplemental Figure 2. Verification of the homozygous *Mi2G02*-expressing *A. thaliana* lines and analysis of their susceptibility to *M. incognita*.

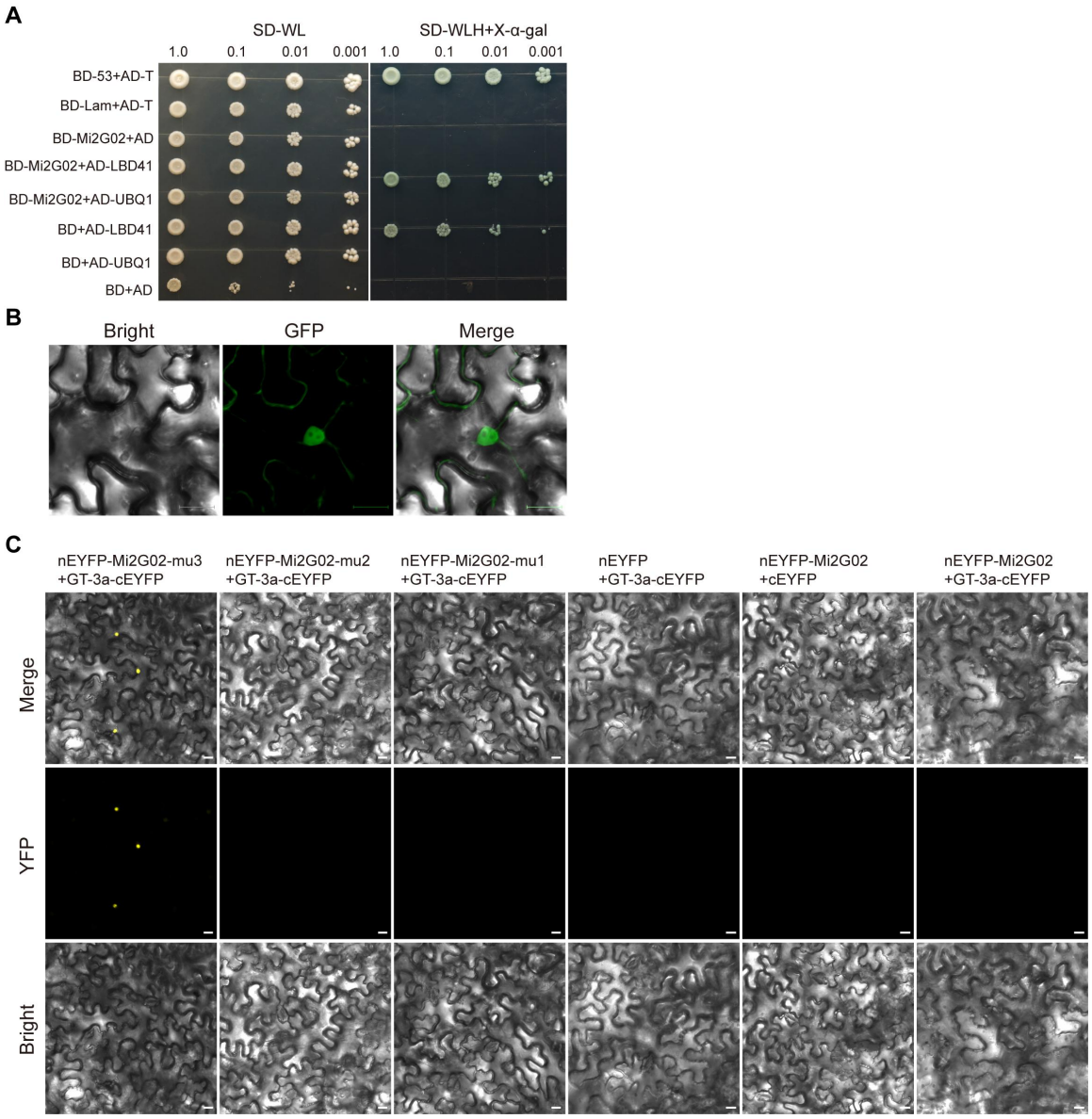
(A) Semi-quantitative RT-PCR was performed to verify *Mi2G02* expression using cDNA of wild-type and two homozygous *Mi2G02*-expressing *A. thaliana* lines (1, Mi2G02-T3-1; 2, Mi2G02-T3-2). *UBP22* (*AT5G10790*) was used as an internal control.

(B) Western-blot using anti-HA antibody was conducted to verify Mi2G02-HA expression in Mi2G02-T3-1 and Mi2G02-T3-2. CBB, coomassie brilliant blue, was used to check protein samples loading.

(C) Root phenotypes of *Mi2G02*-expressing *A. thaliana* lines compared with wild-type plants (WT) under normal growth conditions. The photographs were taken after 14 days. The experiments were repeated three times with similar results, representative pictures are shown. Scale bars, 1 cm.

(D) Two independent nematode infection assays using *Mi2G02*-expressing *A. thaliana* lines. Gall numbers and egg masses were counted at 35 dpi. Values are

presented as means ± SD (n=18 or n=21). Different letters indicate significant differences (P<0.05, one-way ANOVA).

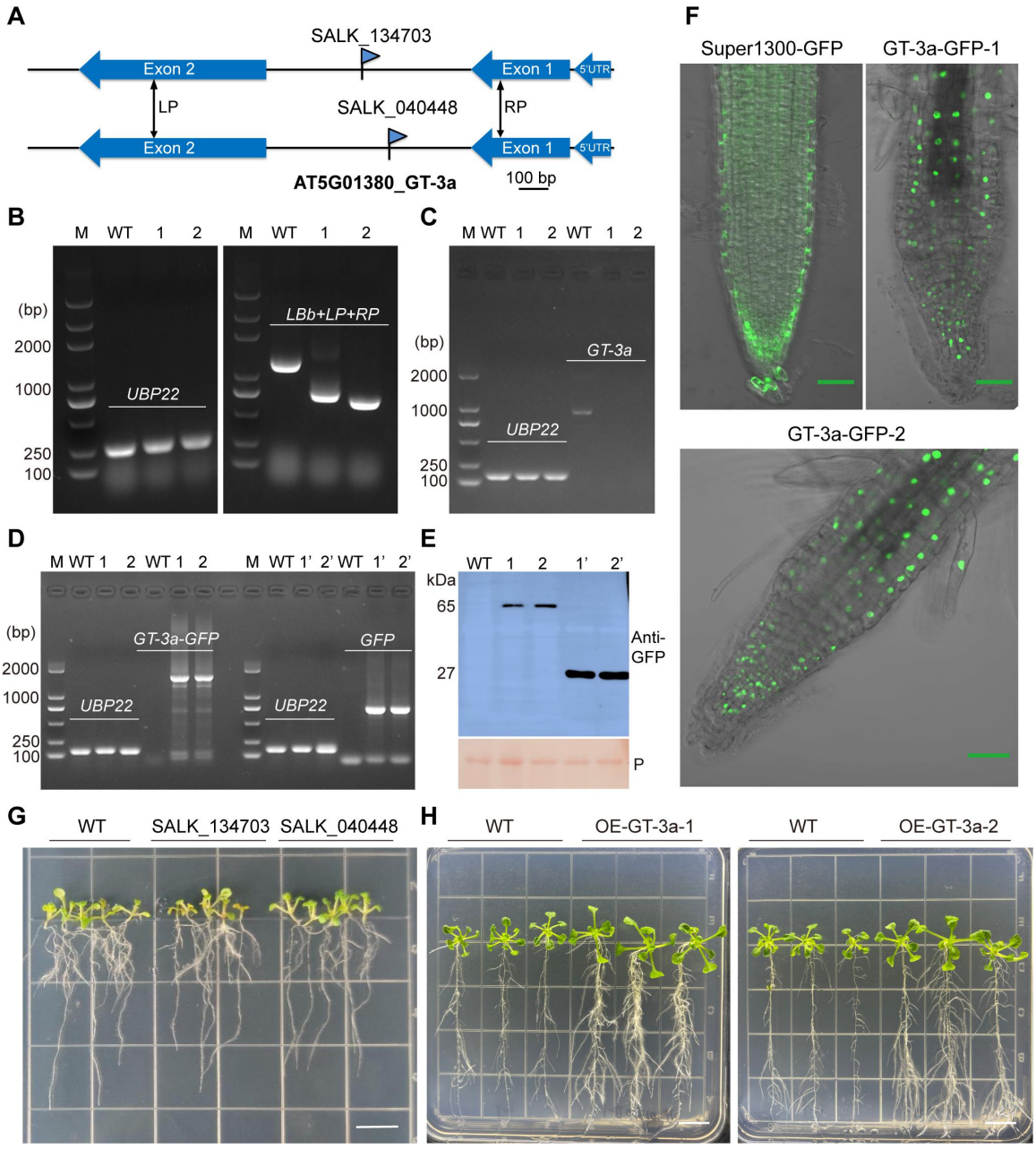


Supplemental Figure 3. Interactions verification between Mi2G02 and GT-3a. **(A)** Autoactivation of LBD41, and GT-3a could not interact with UBQ1 in yeast system. Pairwise yeast two-hybrid (Y2H) tests were performed to investigate the interactions between Mi2G02 and LBD41 or UBQ1. Left column, yeast cell growth carrying the baits in pGBKT7 vector (BD) and preys in pGADT7 (AD) grown on SD/-Trp-Leu (SD-WL) medium indicating successful transformation of the yeast with both plasmids; right column, yeast cell growth on the selective dropout medium (SD/-Trp-Leu-His, SD-WLH) following the addition of 20 mg/ml

X-α-gal indicating protein interaction. Yeast cells containing p53 and SV40 large T-antigen were used as positive control, and yeast cells containing Lamin and SV40 large T-antigen were used as negative control.

(B) Subcellular localization of Mi2G02-ShKT-GFP in *N. benthamiana* leaf cells. Coding sequence of *Mi2G02-ShKT* was constructed into *ProSuper*:GFP (C-terminus GFP) vector. Mi2G02-ShKT-GFP was expressed in *Nicotiana benthamiana* leaf cells. The fluorescence signal was detected at 48 hours after infiltration. Images were captured by confocal microscopy (Zeiss LSM 700, Germany). GFP, green fluorescent protein. Scale bars, 20 μm.

(C) Bimolecular fluorescence complementation (BiFC) experiments demonstrate the interaction between Mi2G02 and GT-3a. *N. benthamiana* leaf epidermal cells were transformed with different combinations of nEYFP- and cEYFP-fusion vectors. Images were obtained 48 h after co-expression. Yellow Fluorescent Protein (YFP) fluorescence signals were observed in the nuclei in leaves co-infiltrated with nEYFP-Mi2G02 and GT-3a-cEYFP. Scale bars, 20 μm.



Supplemental Figure 4. Verification of the homozygous *gt-3a* T-DNA knockout mutants and *GT-3a*-overexpressing *A. thaliana* lines.

(A) Schematic representation of the two *gt-3a* T-DNA insertion mutants.

(B) PCR using genomic DNA was conducted to verify homozygous *gt-3a* KO mutants. 1, SALK_134703; 2, SALK_040448. *UBP22* (*AT5G10790*) was used as an internal control.

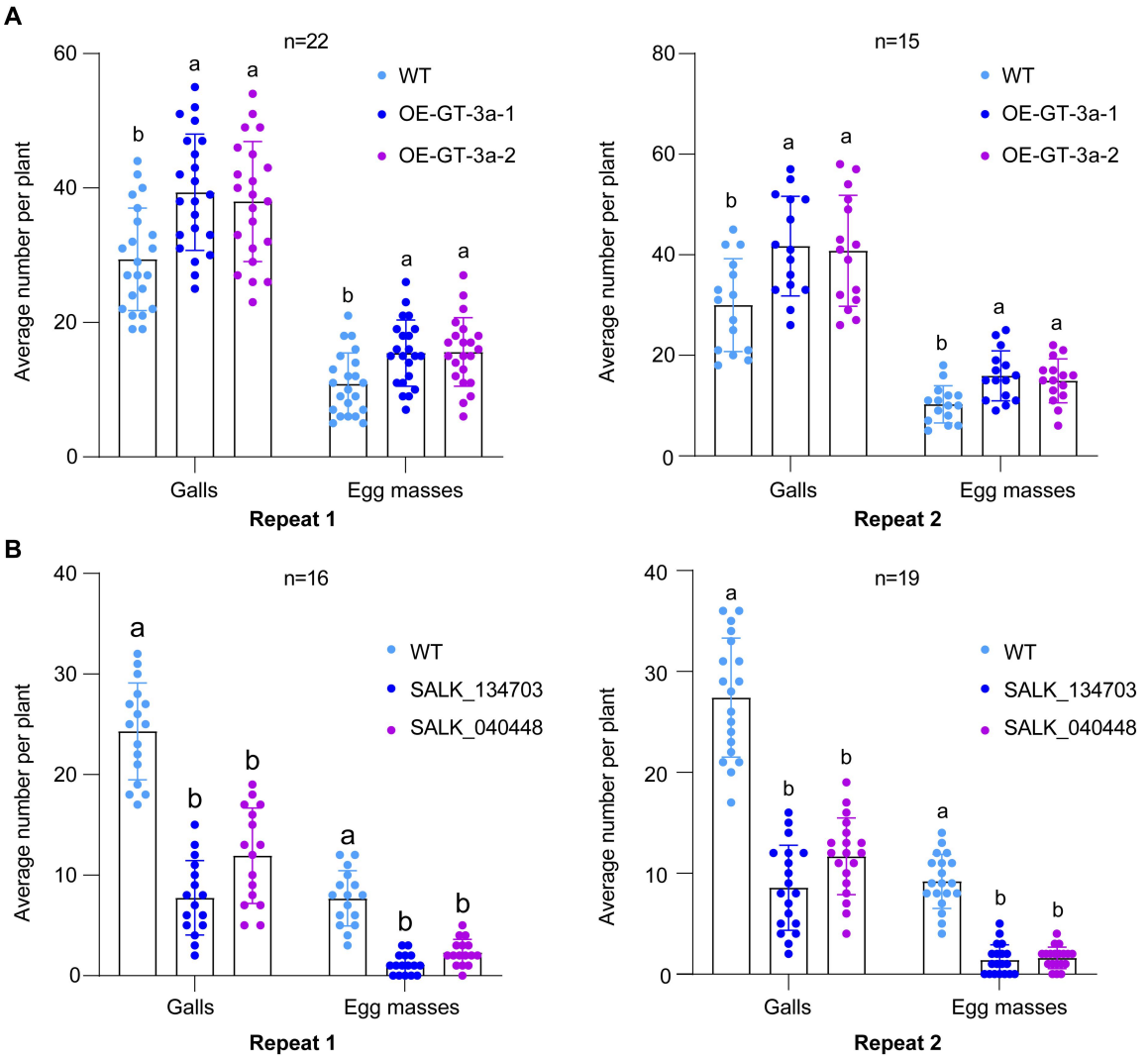
(C) Semi-quantitative RT-PCR using cDNA was conducted to verify homozygous *gt-3a* KO mutants. 1, SALK_134703; 2, SALK_040448. *UBP22* (*AT5G10790*) was used as an internal control.

(D) Semi-quantitative RT-PCR using cDNA was conducted to verify homozygous *GT-3a*- and *GFP*-overexpressing *A. thaliana* lines. 1, OE-GT-3a-1; 2, OE-GT-3a-2; 1', OE-GFP-1; 2', OE-GFP-2. *UBP22* (*AT5G10790*) was used as an internal control.

(E) Western blot using anti-GFP antibodies was conducted to verify GT-3a-GFP expression (predicted about 65 kDa) in OE-GT-3a-1 and OE-GT-3a-2, and GFP expression (predicted about 27 kDa) in OE-GFP-1 and OE-GFP-2. P, ponceau staining indicated loading control.

(F) Localization of the *ProSuper*:GFP and *ProSuper*:GT-3a-GFP fusions under the control of the *Super* promoter in *A. thaliana* roots. Scale bars, 50 μm.

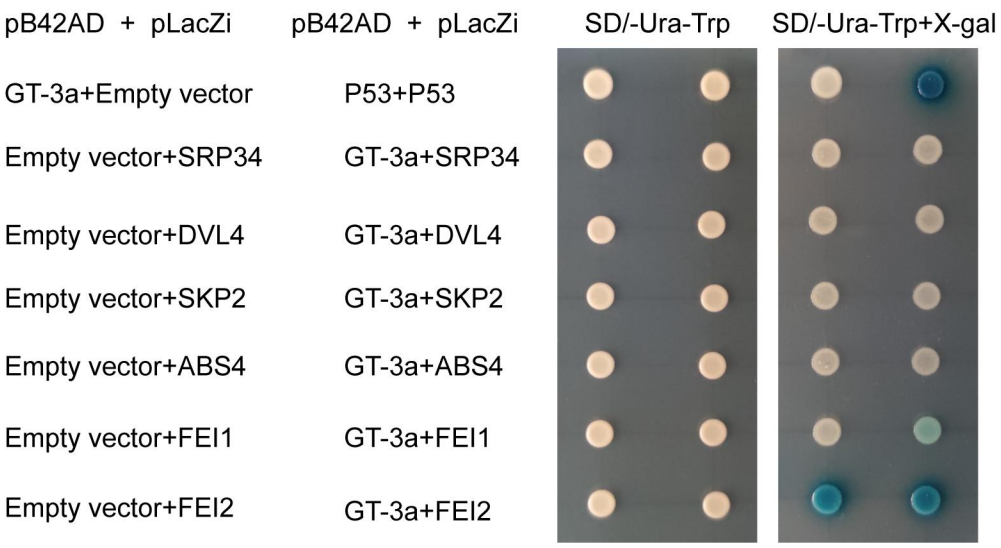
(G and H) Root phenotypes of *gt-3a* KO mutants and *GT-3a*-overexpressing *A. thaliana* lines compared with wild-type plants under normal conditions. The photographs were taken after 14 days. The experiments were repeated three times with similar results, representative pictures are shown. Scale bars, 1 cm.



Supplemental Figure 5. Analysis of the susceptibility of the homozygous *GT-3a*-overexpressing *A. thaliana* lines and *gt-3a* T-DNA knockout mutant lines to *M. incognita*.

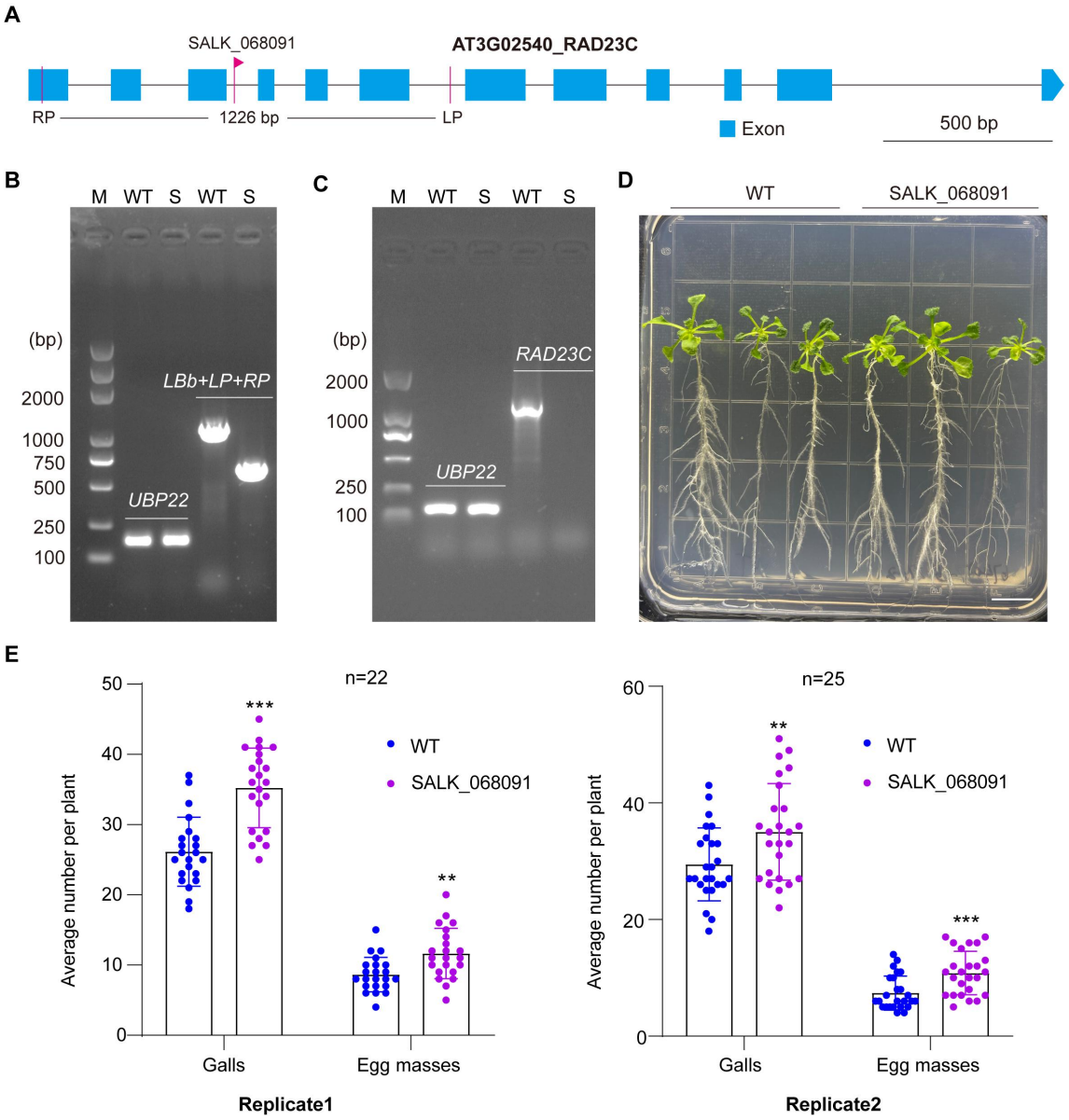
(A) Two independent nematode infection assays using *GT-3a* overexpressing *A. thaliana* lines were performed. Gall numbers and egg masses were counted at 35 dpi. Values are presented as means ± SD (n=22 or n=15). Different letters indicate significant differences (P<0.05, one-way ANOVA).

(B) Two independent nematode infection assays using *gt-3a A. thaliana* mutants. Galls and egg masses were counted at 35 dpi. Values are presented as means ± SD (n=16 or n=19). Different letters indicate significant differences (P<0.05, one-way ANOVA).



Supplemental Figure 6. Yeast one-hybrid (Y1H) assays verify targeting genes by GT-3a.

FEI2 containing -CACGTG- motif showed self-activation in Y1H assays. Y1H results showed that GT-3a could bind to the promoter of *FEI1*, but the activity was low. Y1H experiments showed that GT-3a could not bind to the promoter of *SRP34*, *DVL4*, *SKP2*, *ABS4*. Promoter fragments containing -CACGTG- motif were cloned into pLacZi vector, GT-3a was cloned into pB42AD vector, and then pLacZi vector co-transformed with pB42AD-GT-3a into yeast strain EGY48. The yeast transformants were spotted on the plate SD/-Ura-Trp with or without 20 mg/ml X-gal.



Supplemental Figure 7. Verification of the homozygous *rad23c* T-DNA knockout mutant *A. thaliana* line and phenotype.

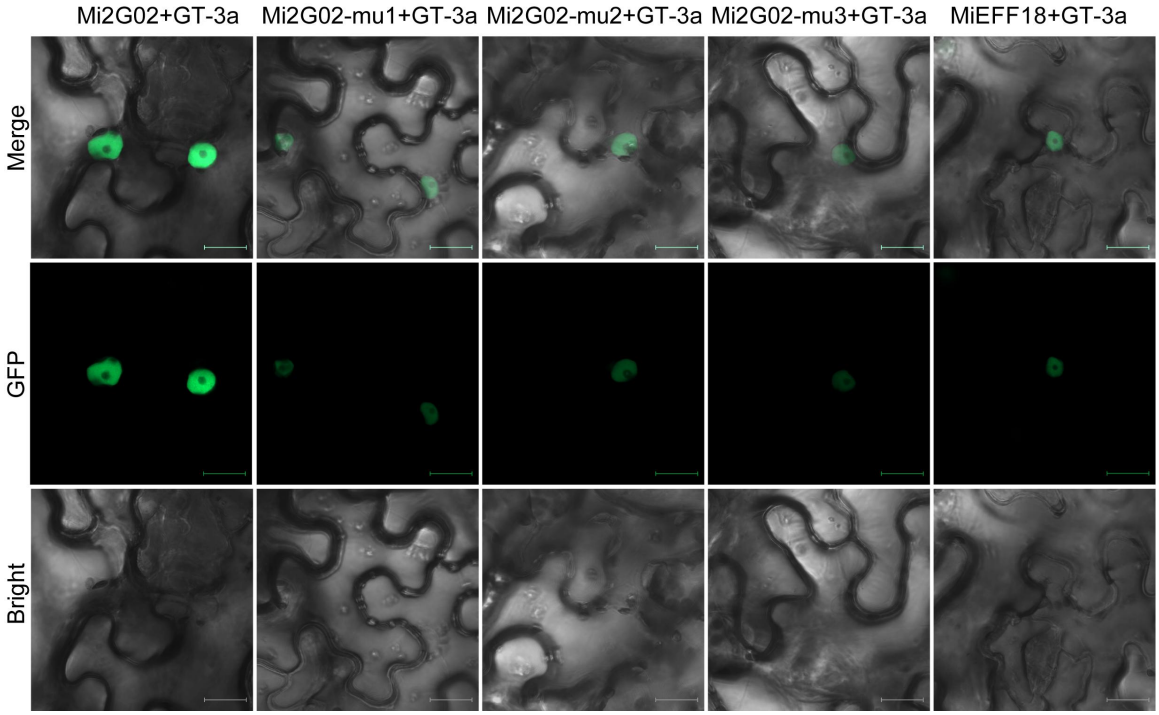
(A) Schematic representation of T-DNA insertion *rad23c* mutants (*SALK_068091*). LP, RP indicate left and right genomic primers. The red triangle indicates the insertion site.

(B) PCR using genomic DNA was conducted to verify homozygous *rad23c* KO mutants. S, SALK_068091. *UBP22* (*AT5G10790*) was used as an internal control.

(C) Semi-quantitative RT-PCR using cDNA was conducted to verify homozygous *rad23c* KO mutants. S, SALK_068091. *UBP22* (*AT5G10790*) was used as an internal control.

(D) The *rad23c* mutants showed no significant difference of root phenotypes when compared with wild-type plants under normal growth conditions. The photographs were taken after 14 days, and a representative picture is shown. Scale bars, 1cm.

(E) The *rad23c* T-DNA knockout mutant is more susceptible than the wild type to *M. incognita*. The *rad23c* KO mutant (*SALK_068091*) was inoculated with nematodes, and the numbers of galls and egg masses were counted 35 days post-inoculation. The data presented are the mean numbers per plant ± SD (n=22 or n=25). Asterisks indicate differences that were significant in two-tailed Student's t tests, $^{**}P<0.01$, $^{***}P<0.001$.



Supplemental Figure 8. Mi2G02 stabilizes the GT-3a-GFP fluorescence intensity.

GT-3a was co-expressed with *Mi2G02* or *GFP* in *N. benthamiana* leaves, and mutant Mi2G02 and MiEFF18 (a nuclear *M. incognita* effector not interacted with GT-3a) were used as controls. The GT-3a-GFP fluorescence was detected with confocal microscopy (LSM700, Zeiss) after 48 h post infiltration. GFP, green fluorescent protein. Scale bars, 20 μm.

Supplemental Table 1. *Arabidopsis thaliana* candidate proteins interacting with Mi2G02 identified by yeast two-hybrid assays.

Genes	Functional annotation in TAIR	Number of clones	In/Out of frame	Screening region
AT5G01380	Homeodomain-like superfamily protein, regulation of transcription	5	In frame	14-210aa (1-323aa)
AT3G52590	*Arabidopsis thaliana* ubiquitin extension protein 1 mRNA involved in embryo development ending in seed dormancy, protein ubiquitination, translation	4	In frame	5-108aa (1-128aa)
AT3G02550	*Arabidopsis thaliana* LOB domain-containing protein, regulation of transcription	4	In frame	91-263aa (1-263aa)
AT1G61170	*Arabidopsis thaliana* mRNA for hypothetical protein	2	In frame	44-251aa (251aa)
AT4G23650	*Arabidopsis thaliana* calcium-dependent protein kinase 6 mRNA	2	In frame	6-333aa (1-529aa)
AT1G09070	*Arabidopsis thaliana* uncharacterized protein mRNA, Arabidopsis thaliana At1g09070/F7G19_6 mRNA	1	In frame	1-277aa (1-324aa)
AT1G28060	*Arabidopsis thaliana* Pre-mRNA-splicing factor 3 mRNA	1	In frame	1-308aa (1-786aa)
AT1G28290	*Arabidopsis thaliana* arabinogalactan protein 31 mRNA	1	In frame	1-308aa (1-786aa)
AT1G31970	*Arabidopsis thaliana* stress response suppressor 1 mRNA	1	In frame	1-250aa (1-537aa)
AT1G56660	MAEBL domain protein	1	Out of frame	
AT2G37190	*Arabidopsis thaliana* ribosomal protein L11 family protein mRNA, complete cds	1	In frame	1-166aa (1-166aa)
AT3G01170	*Arabidopsis thaliana* ribosomal protein L34e superfamily protein mRNA,	1	In frame	134-215aa (1-215aa)
AT3G62240	*Arabidopsis thaliana* RING/U-box domain-containing protein mRNA	1	In frame	415-679aa (1-812aa)
AT3G42660	Ctf4 related nuclear protein. Interacts with LHP1-PRC2 to maintain H3K27 methylation in rapidly dividing cells. EOL1 expression is restricted to rapidly dividing cells.	1	In frame	156-533aa (1-953aa)
AT3G53430	*Arabidopsis thaliana* mRNA for 60S ribosomal Protein L12 –like	1	Out of frame	
AT4G38710	*Arabidopsis thaliana* glycine-rich protein mRNA, translational initiation	1	In frame	1-215aa (1-452aa)
AT5G38840	*Arabidopsis thaliana* SMAD/FHA domain-containing protein mRNA	1	In frame	2-304aa (1-735aa)
AT5G42950	*Arabidopsis thaliana* GYF domain-containing protein	1	In frame	1088-1418aa (1-1714aa)
AT5G53000	PP2A-associated protein with a possible function in the chilling response	1	In frame	1-220aa (1-405aa)
AT5G56290	*Arabidopsis thaliana* putative peroxisomal targeting signal type 1 receptor protein (At5g56290) mRNA	1	Out of frame	

Supplemental Table 2. *Arabidopsis thaliana* candidate genes targeted by the GT-3a protein.

Genes	Function predication in TAIR
AT1G02840	ATSRP34, Splicing Factors (SR proteins).
AT1G13245	DVL4, negative regulation of cell population proliferation.
AT1G21410	AtSKP2, positive regulation of cell division.
AT1G31420	FEI1. Encodes a plasma membrane localized leucine-rich repeat receptor kinase that is involved in cell wall elongation.
AT1G58340	ABS4, encodes a plant MATE transporter that is localized to the Golgi complex and small organelles and is involved in determining the rate of organ initiation.
AT2G35620	FEI2. Encodes a plasma membrane localized leucine-rich repeat receptor kinase that is involved in cell wall elongation.
AT3G02540	RAD23C. Encodes a member of the RADIATION SENSITIVE23 (RAD23) family, RAD23 proteins play an essential role in the cell cycle.
AT5G16750	TOZ. Encodes a nucleolar localized WD-40 repeat protein that is preferentially expressed in dividing cells and is required for regulated division planes and embryo development.
AT5G56270	WRKY2. Encodes WRKY transcription factor 2, a zinc-finger protein. In wrky2 mutants, egg cells polarize normally but zygotes fail to reestablish polar organelle positioning from a transient symmetric state, resulting in equal cell division and distorted embryo development.

Supplemental Table 3. Primers used in this research.

Primers for real-time quantitative PCR (RT-qPCR)	
Primer name	Primer sequences (5'-3')
QPCR-GT-3a-F	GGAAGTCGTGGCTGCTAAGAT
QPCR-GT-3a-R	GAATGGGAACTGCTGCCTAAT
QPCR-RAD23C-F	CAGAACCCAAACTTGATGCG
QPCR-RAD23C-R	GCATTCCCGCCGCCATTT
QPCR-TOZ-F	ATGGTTCTTTCATCGCCTGC
QPCR-TOZ-R	GACTCGCCTTCAATCGTGG
QPCR-Mi2G02-F	ACGGCATCAACAATAACAACACC
QPCR-Mi2G02-R	CACATTCGGTTGTCTCGGTTG
GAPDH-F	CGTGCAGCGGTTGAGAAGGA
GAPDH-R	GCGTCCGTGGGTGGAATCAT
Primers for vectors of luciferase complementation assay	
Primer name	Primer sequences (5'-3')
nluc-Mi2G02-F	acgggggacgagctcggtaccATGGAATGTAGCGGAGATTGTTC
nluc-Mi2G02-R	cgcgtacgagatctggtcgac CAATTTAGCATGAATCTTAAC
cluc-GT-3a-F	tacgcgtcccggggcggtaccATGGACCGACGTAACCCTTTC
cluc-GT-3a-R	acgaaagctctgcaggtcgacTCAGAAACCTTGATTATGATGATC
Primers for vectors of subcellular localization assay	
Primer name	Primer sequences (5'-3')
Mi2G02-GFP-F	aatactagtggatccggtaccATGGAATGTAGCGGAGATTGTTC
Mi2G02-GFP-R	gcccttgctcaccatggtaccCAATTTAGCATGAATCTTAACTTTCGG
ShkT-GFP-F	aatactagtggatccggtaccATGGAATGTAGCGGAGATTGTTC
ShkT-GFP-R	gcccttgctcaccatggtaccATGACAAACTTTACATTTCTTTGGA
Primers for vectors of *Arabidopsis thaliana* transformation	
Primer name	Primer sequences (5'-3')
GT-3a_super_F	aatactagtggatccggtaccATGGACCGACGTAACCCTTTC
GT-3a_GFP_R	gcccttgctcaccatggtaccGAAACCTTGATTATGATGATCATCATT
Mi2G02-HA-F	ctgcaggggcccggggtcgacATGGAATGTAGCGGAGATTGTTC
Mi2G02-HA-R	aacatcgtatgggtaggtaccCAATTTAGCATGAATCTTAACTTTCGG
Primers for identifying transgenic *Arabidopsis thaliana*	
Primer name	Primer sequences (5'-3')
GFP-F	ATGGTGAGCAAGGGCGAG
GFP-R	ACTTGTACAGCTCGTCCATGC
Mi2G02-F	ATGGAATGTAGCGGAGATTGTTCT
Mi2G02-HA-R	TCAAGCGTAATCTGGAACATCGT
GT-3a-F	ATGGACCGACGTAACCCTTTC
GT-3a-R	CCTATTAAGCAAAGCATCGATGAGAG

GT-3a-F	ATGGACCGACGTAACCCTTTC
GFP-R	ACTTGTACAGCTCGTCCATGC
Primers for identifying T-DNA knockout mutant of *gt-3a*	
Primer name	Primer sequences (5'-3')
SALK_134703_LP	TTCACCGTTTGCCTCATAAAC
SALK_134703_RP	GAAGTCGTGGCTGCTAAGATG
SALK_040448_LP	TTCACCGTTTGCCTCATAAAC
SALK_040448_RP	GAAGTCGTGGCTGCTAAGATG
LBb1.3	ATTTTGCCGATTTCGGAAC
Primers for constructing GUS vector	
Primer name	Primer sequences (5'-3')
PBI101-GT-3a-F	cttgcatgcctgcaggtcgacGATGGGAAATAAAAAGGATGATGAG
PBI101-GT-3a-R	ggactgaccacccggggatccTATTTGGAATCGAATTGTCTTTGTG
Primers for constructing prokaryotic expression vector	
Primer name	Primer sequences (5'-3')
PET30a-GT-3a-F	gccatggctgatatcggatccATGGACCGACGTAACCCTTTC
PET30a-GT-3a-R	tgcggccgcaagcttgtcgacGAAACCTTGATTATGATGATCATCATT
Primers for identifying T-DNA knockout mutant of *rad23c*	
Primer name	Primer sequences (5'-3')
SALK_068091-LP	ATTTTCGTACCGTCATGCAAC
SALK_068091-RP	GCAGCCAACTCTTCATTCTTG
LBb1.3	ATTTTGCCGATTTCGGAAC
Primers for identifying gene expression using cDNA	
Primer name	Primer sequences (5'-3')
GT-3a-CDS-F	ATGGACCGACGTAACCCTTTC
GT-3a-CDS-R	TTAGAAACCTTGATTATGATGATCATCA
Mi2G02-CDS-F	ATGGAATGTAGCGGAGATTGTTC
Mi2G02-CDS-R	TTACAATTTAGCATGAATCTTAACTTTCG
Mi2G02-CDS-F	ATGGAATGTAGCGGAGATTGTTC
Mi2G02-CDS-R	TTACAATTTAGCATGAATCTTAACTTTCG
Primers for constructing pSAT5 vector (host-derived RNAi)	
Primer name	Primer sequences (5'-3')
Mi2G02-F1-NcoI-XbaF	CATGCCATGGTCTAGACTTATTCAACCGAGACAACCGA
Mi2G02-F1-XhoR	CCGCTCGAGTCATCTTCTTTGCGTCTTTGAA
Mi2G02-F2-PstF	AACTGCAGTCATCTTCTTTGCGTCTTTGAA
Mi2G02-F2-KpnR	GGGGTACCCTTATTCAACCGAGACAACCGA
Primers for vectors of bimolecular fluorescence complementation	
Primer name	Primer sequence (5'-3')
Mi2G02-CE-SalF	ACGCGTCGAC ATGGAATGTAGCGGAGATTGTTC
Mi2G02-CE-XmaR	TCCCCCCGGGCAATTTAGCATGAATCTTAAC

AT3G02550-CE-SalF	ACGCGTCGAC ATGCGGATGAGCTGTAATGG
AT3G02550-CE-XmaR	TCCCCCCGGG GAGCATAAGCTCAGTCTTAC
AT3G52590-CE-SalF	ACGCGTCGAC ATGCAGATCTTCGTGAAAAC
AT3G52590-CE-XmaR	TCCCCCCGGG CTTGATCTTCTTCTTAGGCC
GT-3a-CE-SalF	ACGCGTCGAC ATGGACCGACGTAACCCTTTC
GT-3a-CE-XmaR	TCCCCCCGGG GAAACCTTGATTATGATGATC
Primers for vectors of yeast two-hybrid assays	
Primer name	Primer sequence (5'-3')
AD-52590-F	gccatggaggccagtgaattcATGCAGATCTTCGTGAAAACCTT
AD-52590-R	cagctcgagctcgatggatccCTACTTGATCTTCTTCTTAGGCCTCA
AD-Mi2G02-F	gccatggaggccagtgaattcATGGAATGTAGCGGAGATTGTTC
AD-Mi2G02-R	cagctcgagctcgatggatccTTACAATTTAGCATGAATCTTAACTTTCG
AD-ShKT-F	gccatggaggccagtgaattcATGTATAAATGTGAGGATAGAAGTGAATTT
AD-ΔShKT-F	gccatggaggccagtgaattcATGCATAGTTCTGGTGAAGAACCTAA
AD-ShKT-R	cagctcgagctcgatggatccTTAATGACAAACTTTACATTTCTTTGGA
AD-02550-F	gccatggaggccagtgaattcATGCGGATGAGCTGTAATGGA
AD-02550-R	cagctcgagctcgatggatccTTAGAGCATAAGCTCAGTCTTACACG
AD-GT-3a-F	gccatggaggccagtgaattcATGGACCGACGTAACCCTTTC
AD-GT-3a-R	cagctcgagctcgatggatccTTAGAAACCTTGATTATGATGATCATCA
AD-GT-3a-DB-F	gccatggaggccagtgaattcATGCCACAGTGGAGCATAGAGG
AD-GT-3a-DB-R	cagctcgagctcgatggatccTTAAATCGACTGAATCTCATTGTAGAAT
AD-GT-3a-ΔDB-F	gccatggaggccagtgaattcATGTTTGAAGCAAGAATGCAAAGA
Mi2G02-BDF	atggccatggaggccgaattcGAATGTAGCGGAGATTGTTCTATAGAG
Mi2G02-BDR	ccgctgcaggtcgacggatccTTACAATTTAGCATGAATCTTAACTTTCG
BD-GT-3a-F	atggccatggaggccgaattcATGGACCGACGTAACCCTTTC
BD-GT-3a-R	ccgctgcaggtcgacggatccTTAGAAACCTTGATTATGATGATCATCA
BD-GT-3a-DB-F	atggccatggaggccgaattcATGCCACAGTGGAGCATAGAGG
BD-GT-3a-DB-R	ccgctgcaggtcgacggatccTTAAATCGACTGAATCTCATTGTAGAAT
BD-GT-3a-ΔDB-F	atggccatggaggccgaattcATGTTTGAAGCAAGAATGCAAAGA
Primers for vectors of yeast one-hybrid assays	
Primer name	Primer sequence (5'-3')
PLaczi-SRP34-F	ATCTGTCGACCTCGAGGAACCTATCTAACAACAAGCC
PLaczi-SRP34-R	GAGCACATGCCTCGAGGTCCAAAGTCAAGATTCAAAC
PLaczi-DVL4-F	ATCTGTCGACCTCGAGGTAATGAGAGACTTGCAACTTC
PLaczi-DVL4-R	GAGCACATGCCTCGAGGAGGATACAAAGAAGAAGAGAG
PLaczi-SKP2-F	ATCTGTCGACCTCGAGCAAGTCATTTTGGCTTGGAAT
PLaczi-SKP2-R	GAGCACATGCCTCGAGTGAAGTGTTTCACAGAGCTAG
PLaczi-FEI1-F	ATCTGTCGACCTCGAGGATTAGGCTTAGCTAAGGTCT
PLaczi-FEI1-R	GAGCACATGCCTCGAGGAAGAAGATGACTAATTTGAGC
PLaczi-ABS4-F	ATCTGTCGACCTCGAGCTTCTCACAGACTTATTTGGTG

PLaczi-ABS4-R	GAGCACATGCCTCGAGGATTCTTAAGAGGAGTAAACTGG
PLaczi-FEI2-F	ATCTGTCGACCTCGAGCGCAATAACGATGTTTTAACT
PLaczi-FEI2-R	GAGCACATGCCTCGAGTGAAGAATAAGACAGAGGAAG
PLaczi-RAD23C-F	ATCTGTCGACCTCGAGCCTATCTGCTATCATTTAATAAGTG
PLaczi-RAD23C-R	GAGCACATGCCTCGAGCTCACCGTCGAAATTCTCTTC
PLaczi-TOZ-F	ATCTGTCGACCTCGAGGAGATCATACAGAATCGAGATTC
PLaczi-TOZ-R	GAGCACATGCCTCGAGCATGAACTATATAATTGGCCCA
PLaczi-WRKY2-F	ATCTGTCGACCTCGAGGTTAGAGATCACCTGTGTTAC
PLaczi-WRKY2-R	GAGCACATGCCTCGAGGAGAAGAGGAATCTTCGAAAG
pB42AD-01380-F	TGCCTCTCCCGAATTCATGGACCGACGTAACCC
pB42AD-01380-R	CGAGTCGGCCGAATTCTAGAAACCTTGATTATGATGATCATC