Supplemental Information

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Figures S1-S10

Supporting Information – Figure Legends

Figure S1. Phenotypic differences in Arabidopsis Col-0 plants expressing JAZ1 or JAZ9 lacking a functional Jas domain. (*A, B*) Twelve-day-old seedlings grown on MS media supplemented with 10 μM MeJA. Images were collected by scanning growth plates (*A*), and root growth was quantified using ImageJ software (*B*). Data are the means from 15 plants; error bars represent SD. Letters on columns indicate statistically significant differences ($p < 0.01$, Tukey's HSD test). (*C*) Western blot analysis of the stability of JAZ1 and JAZ9 in transgenic Arabidopsis plants after one-hour treatment with coronatine (COR) or methyl JA (MeJA) treatment. 3×HA:JAZ fusion proteins were detected by western blot using a monoclonal antibody against the HA tag; CBB = coomassie brilliant blue staining of PVDF membranes.

Figure S2. JAZ1ΔJas interacts with MYC2 *in vitro*. Ten micrograms of purified recombinant GST:MYC2 protein were used to pull down HA:JAZ proteins from yeast lysates. EV: Lysate from yeast carrying empty vector. Five percent of the input and 20% of the pull-down material were separated by SDS-PAGE, and HA:JAZ and GST:MYC2 proteins were detected using anti-HA antibody and anti-GST antibody, respectively.

Figure S3. Deletion of the Jas domain affects sub-cellular localization of JAZ1 and JAZ9. Overlay of bright-field and fluorescent images of YFP:JAZ1, YFP:JAZ9, JAZ1ΔJas, JAZ9ΔJas, and YFP transiently expressed in *N. tabacum* epidermal cells. Inserts are portions of images enlarged to show nuclei. Scale bar = 10 μm.

Figure S4. YFP:JAZ1ΔJas and YFP:JAZ9ΔJas are expressed at similar levels during transient expression in *N. tabacum*. Western blot analysis of the expression levels of YFP:JAZ1ΔJas (A), YFP:JAZ9ΔJas (B) and the YFP control (C). The proteins were detected using a polyclonal antibody against GFP. CBB = Coomassie brilliant blue.

Figure S5. Subcellular localization of COI1 and NINJA. Overlay of bright-field and fluorescent images of *YFP:COI1* and *YFP:NINJA* transiently expressed in *N. tabacum* epidermal cells. YFP signal was detected inside the nucleus. Images were taken 24-30 hours post-inoculation using laser scanning confocal microscopy. Scale bar = 10 μm.

Figure S6. JAZ9 interacts with the N terminus of MYC2. *(A)* Diagram of MYC2 and truncated derivatives. AD = transcriptional activation domain, BHLH = basic helix loop helix region. *(B)* Yeast two hybrid assay testing the interaction between JAZ9 and specific regions of MYC2. Yeast cultures cotransformed with MYC2:AD derivatives, and either JAZ9:BD (top panel), or GAL4BD (bottom panel) were spotted on –LWH media. Protein interactions are indicated by colony growth.

Figure S7. Relative transcript levels of *MYC2*, *MYC3* and *MYC4* in Col-0 plants. Total cellular RNA was extracted from leaves of 18-day-old *Arabidopsis* Col-0 plants and used for qRT-PCR analysis. Transcript levels of *MYC2*, *MYC3* and *MYC4* were normalized against the control gene transcript *PP2AAC* (*At1G13320*) using the $2^{-\Delta Ct}$ method (1). Shown are the means of four biological replicates. Error bars indicate standard errors of the means. Asterisks indicate

significant difference in transcript level compared to *MYC2* as determined by Student's t-test ($P<0.05$).

Figure S8. Localization of YFP:JAZ9 and its mutants in transgenic Arabidopsis seedlings. Fluorescent images of YFP:JAZ9 carrying specific alanine substitutions in the Jas motif expressed in transgenic Arabidopsis seedlings. Second generation transgenic seedlings were used. Scale bar = 50 μm.

Figure S9. Forced targeting of JAZ9ΔJas to the nucleus is not sufficient to confer JA-insensitivity. (*A*) Bright-field and fluorescent image overlay of mCherry (mCH), mCH:JAZ9, mCH:JAZ9ΔJas, and mCH:JAZ9ΔJas-NLS stably expressed in transgenic Arabidopsis Col-0 plants. mCH:JAZ9ΔJas-NLS is contains a VirD2-NLS at the C-terminal end of JAZ9ΔJas. (*B*) Pictures of twelve-day-old transgenic Arabidopsis seedlings grown on 10 μM meJA. Scale bar = 10 mm. (*C*) Quantification of root length. Growth plates were scanned to capture images, and root lengths were measured using ImageJ software. Data are the means from 15 plants; error bars represent SD. Letters on columns indicate statistically significant differences ($p < 0.01$, Tukey's HSD test).

Figure S10. Transgenic expression of YFP:JAZ9-RK^{Jas6,7}AA causes JA-insensitive phenotypes. (*A*) Pictures of ten-day-old Arabidopsis seedlings grown on 10 μM Me-JA. Scale bar = 5 mm. (*B*) Pictures of 14-day-old seedlings grown on 10 μM meJA. YFP:JAZ9-RK^{Jas6,7}AA (Jas6,7) and *coi1-30* were insensitive to MeJA treatment as indicated by green cotyledons and leaves compared to Col-0, YFP, YFP:JAZ9, and YFP:JAZ9-KRK$^{Jas16-18}$AAA (Jas16-18) seedlings. Scale bar = 10 mm. (*C*) Symptom development on leaves three days post-inoculation with 1x 10^6 CFU/ml *Pst* DC3000. Scale bar = 1.5 cm.

Supplemental Materials and Methods

Chemicals and reagents used: Methyl-jasmonate, coronatine, complete protease inhibitor cocktail for plant cell and tissue extracts, antibiotics, and acetosyringone (Sigma, St. Luois, MO), 26S proteasome inhibitor MG132 (Cayman Chemical Co., Ann Arbor, MI), rabbit polyclonal antibody to GFP (Ab290, AbCam, Cambridge, MA), HRP-conjugated goat anti-rabbit antibody (Pierce, Rockford, IL).

Site-directed mutagenesis. The DNA sequences of selected amino acid residues in the Jas domain of JAZ9 (At1G70700) were mutated to alanine codons using the Quick-Change II site-directed mutagenesis kit (Agilent, Santa Clara, CA). Mutagenesis was confirmed by DNA sequencing, and western blot was performed to confirm the production of the mutant proteins when coexpressed with COI1 (At2G39940) or MYC2 (At1G32640) in yeast-two-hybrid assays.

Generation of plant expression constructs. The coding sequences of *MYC2, JAZ1, JAZ9* and selected *JAZ* mutants were cloned into pENTR4 (Invitrogen, Grand Island, NY), and transferred by LR recombination into the binary expression vector pEarleyGate 104 (2) to generate a *35S:YFP:JAZ9* fusion. *35S:CFP:MYC2* fusions were generated by recombination of the *MYC2* coding sequence into pVKgw (3). *35S:mCherry:JAZ9:NLS* constructs were generated by ligating the coding sequence of *JAZ9* or *JAZ9ΔJas* into pSAT6-mCherry with or without VirD2NLS (4). The correct reading frame for all constructs was confirmed by sequencing. All constructs were introduced into *Agrobacterium tumefaciens* (GV3101). GV3101 clones containing the gene fusion constructs were selected on LB medium containing rifampicin, kanamycin and gentamycin antibiotics.

Transient gene expression in *N. tabacum.* Single *Agrobacterium* colonies containing JAZ9 constructs were selected and cultured over-night at 30 °C in LB medium containing appropriate antibiotics. *Agrobacterium tumefaciens (GV3101)* cells were harvested from overnight cultures by centrifugation at 3,500 rpm for ten minutes, washed in sterile water and resuspended to OD600 = 0.2 in infiltration buffer (10 mM MES pH 5.8; 10 mM $MgCl_2$; 0.2 % sucrose; 300 μM acetosyringone). Cultures were syringe-inoculated into mature leaf tissue of *Nicotiana tabacum*. Infiltrated plants were returned to previous growing conditions and expression was analyzed by

confocal microscopy at 24-30 hours after inoculation. Fusion protein expression was analyzed by western blot.

Generation of transgenic Arabidopsis. Arabidopsis Col-0 plants were transformed using the standard floral dip protocol (5). Ten to fifteen BASTA resistant T1 seedlings were identified, then transplanted to separate pots and grown to maturity. The T2 generation seedlings were again selected on MS-agar plates containing 10 mg/L BASTA, and screened for gene expression level by RT-PCR (using *YFP*- and *JAZ9*-specific forward and reverse primers, respectively) and western blot using a polyclonal antibody against GFP.

Confocal microscopy. Transiently expressed fusion proteins and all transgenic Arabidopsis lines were analyzed using a Zeiss 510 Meta ConfoCor3 laser scanning confocal microscope. Microscopic examination of all plant tissue samples was conducted using an EC Plan-Neofluar 40x/1.30 Oil objective with 2X and 4X digital zoom applied with AIM software (Zeiss). For detection of YFP, samples were excited with an argon laser at 10% strength, 514nm; the optical pathway included an HFT 458/514 beam splitter, BP520-555IR band pass filter and NFT 515 and 595 long pass filters. For detection of CFP fusion proteins, samples were excited with an argon laser at 30-40% strength, 458nm; the optical pathway included an HFT 458/514 beam splitter, BP465-510IR band pass filter and NFT 515 and 595 long pass filters. For detection of mCherry fusion proteins, samples were excited with a HeNe 543 laser at 30%. The optical pathway included HFT 488/543 beam splitter, NFT 635 and NFT 545 long pass filters, and a BP 560-615 band pass filter. For colocalization experiments, dual channel, sequential imaging was used to capture images.

Yeast-two-hybrid (Y2H). GAL4 system (Clontech, Mountainview, CA) was used for testing interaction of MYC2 with JAZ1ΔJas or JAZ9ΔJas, and also interaction of truncated MYC2 with JAZ9. Cotransformed yeast were grown on SD glucose (-LW) for selective propagation of cultures and on SD glucose (-LWH) for determining protein interactions. Y2H plates were supplemented with 15 mM 3-amino-1,2,4-triazole (3-AT) to eliminate reporter gene auto-activation LexA system (Clontech, Mountainview, CA) was used for all other Y2H experiments. The coding sequence of *COI1* was cloned into the Y2H bait vector pGILDA (Clontech, Mountainview, CA) using *XmaI* and *XhoI* restriction enzyme recognition sequences previously added to the 5' and 3' end of the *COI1* gene, respectively, creating a *LexA:COI1* fusion. The

coding sequence of *JAZ9*, and site-directed mutants, were cloned into the Y2H prey vector pB42AD (Clontech), creating AD:JAZ9 fusions. Individual *COI1* constructs were co-transformed with *JAZ9* or *JAZ9* mutant constructs into yeast (*S. cerevisae*) strain EGY48 (p8opLacZ) using the frozen-EZ yeast transformation II kit (Zymo Research, Irvine, CA). Transformants were selected on SD-glucose medium (BD Biosciences, San Jose, CA) supplemented with –Ura/-Trp/–His drop-out solution (BD Biosciences).

To detect the interaction between COI1 and JAZ9 or JAZ9 mutant proteins, yeast transformants that had been selected in SD-Glu –U/W/H medium were resuspended in sterile water, and 10 µl of each suspension was spotted onto inducing media (SD-Galactose/Raffinose –U/W/H; BD Biosciences) supplemented with 80 µg ml^{-1} X-Gal and 10 µM coronatine (Sigma). The Y2H assays were incubated in the dark at 30°C; pictures were taken after approximately 48 hours. Induced clones were analyzed to confirm protein expression by western blotting using epitope-specific antibodies. Interaction between JAZ9, JAZ9 mutants, and MYC2 was conducted as described above; however, for these Y2H assays, JAZ9 and mutants were fused to the DNA binding domain in pGILDA, and MYC2 was fused to the transcriptional activation domain pB42AD to eliminate auto-activation of the *LacZ* reporter gene.

Growth inhibition assays. Arabidopsis wild-type Col-0, heterozygous *coi1-30* plants and transgenic seedlings expressing *YFP:JAZ9* (or mutant variations) under the CaMV 35S promoter were surface sterilized, cold-stratified and germinated on MS agar media containing no treatment or 10 µM methyl jasmonate. Seedlings were grown for 10-12 days in long day light conditions (100 µE light intensity; 16L:8D). Pictures of seedlings were taken and plates were then scanned to high-resolution images. Root lengths were analyzed using ImageJ software (http://rsbweb.nih.gov/ij/).

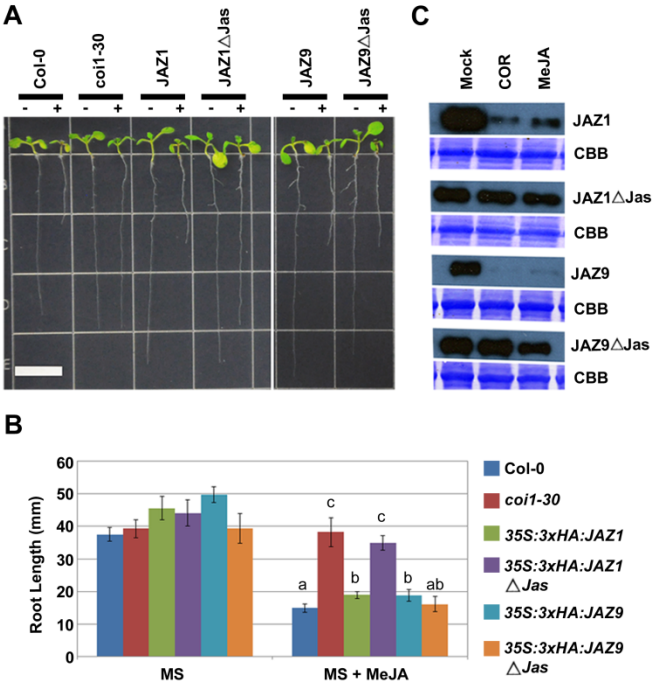
Bacterial infection assays. For plant growth, all seedlings were surface sterilized, cold-stratified and germinated on plates containing MS agar with appropriate selection reagents in the media. For selection of *coi1-30* mutants, seeds from the segregating population were germinated on media supplemented with 50 µM methyl jasmonate, grown for five to seven days, and then JA-insensitive mutants were transplanted into pots containing standard Arabidopsis potting soil. Transgenic Arabidopsis expressing the *YFP:JAZ9* (or JAZ9 mutant variations) were selected for the presence of the transgene by selection on media supplemented with 10 mg/L BASTA and

then transplanted to soil. Wild-type Col-0 seedlings were germinated on blank MS agar media; all seedlings were germinated and transplanted simultaneously. After transplanting, the seedlings were grown under short day light conditions (100 μE light intensity; 12L:12D) for approximately five weeks. Syringe-inoculation of *Pseudomonas syringae pv. tomato* DC3000 was conducted using the standard laboratory protocol (6). Symptom development and bacterial multiplication were analyzed three days post-inoculation.

Supplemental References

1. Livak KJ & Schmittgen TD (2001) Analysis of Relative Gene Expression Data Using Real-Time Quantitative PCR and the 2−ΔΔCT Method. *Methods* 25(4):402-408.
2. Earley KW*, et al.* (2006) Gateway-compatible vectors for plant functional genomics and proteomics. *The Plant Journal* 45(4):616-629.
3. Tian M*, et al.* (2011) 454 Genome Sequencing of Pseudoperonospora cubensis Reveals Effector Proteins with a QXLR Translocation Motif. *Molecular Plant-Microbe Interactions* 24(5):543-553.
4. Lee L-Y, Fang M-J, Kuang L-Y, & Gelvin S (2008) Vectors for multi-color bimolecular fluorescence complementation to investigate protein-protein interactions in living plant cells. *Plant Methods* 4(1):24.
5. Clough SJ & Bent AF (1998) Floral dip: a simplified method forAgrobacterium-mediated transformation ofArabidopsis thaliana. *The Plant Journal* 16(6):735-743.
6. Katagiri F, Thilmony R, & He SY (2002) The Arabidopsis Thaliana-Pseudomonas Syringae Interaction. *The Arabidopsis Book*:e0039.

Figure S1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coi1-30
JAZ1
JAZ1△Jas
JAZ9
JAZ9△Jas
- +
C
Mock
COR
MeJA
JAZ1
CBB
JAZ1△Jas
CBB
JAZ9
CBB
JAZ9△Jas
CBB
B
Root Length (mm)
60
50
40
30
20
10
0
a
c
b
c
b
ab
MS
MS + MeJA
Col-0
coi1-30
35S:3xHA:JAZ1
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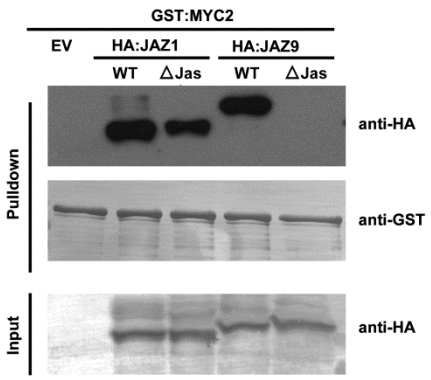
35S:3xHA:JAZ9 △Jas

Figure S2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EV
HA:JAZ1
HA:JAZ9
WT
ΔJas
WT
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anti-GST
Input
anti-HA

Figure S3

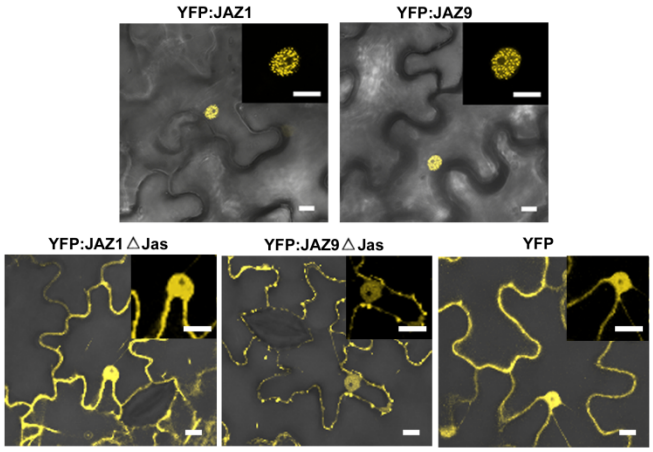
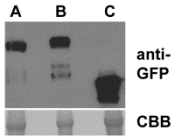


Figure S4



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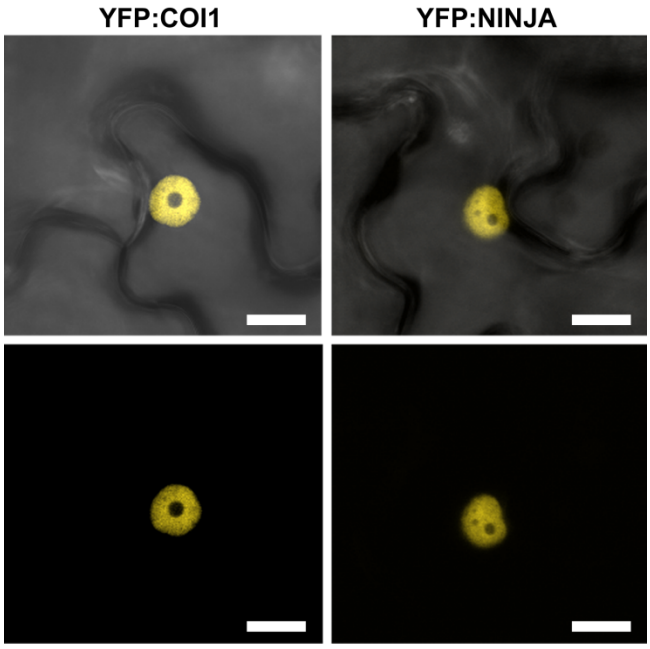

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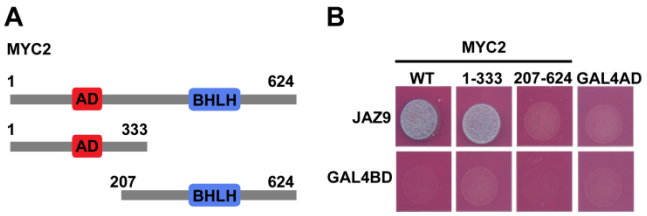
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AD
BHLH
624
1
AD
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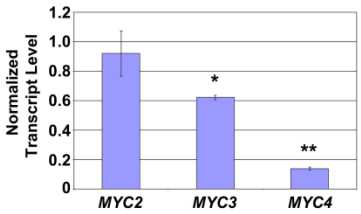
207-624
GAL4AD
JAZ9
GAL4BD

Figure S7


Normalized Transcript Level
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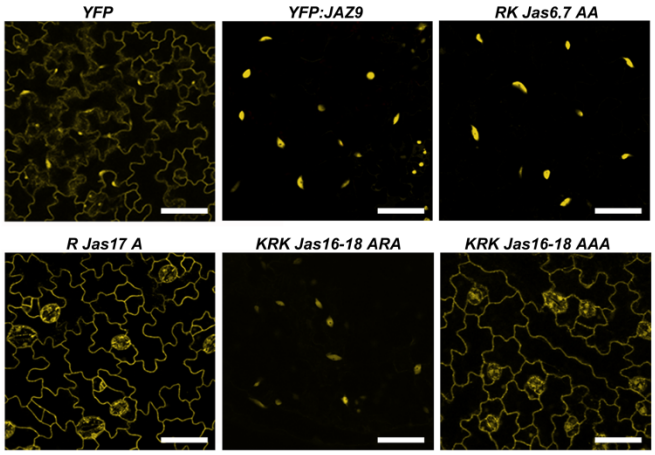
0.8
0.6
0.4
0.2
0
*
**
MYC2
MYC3
MYC4

Figure S8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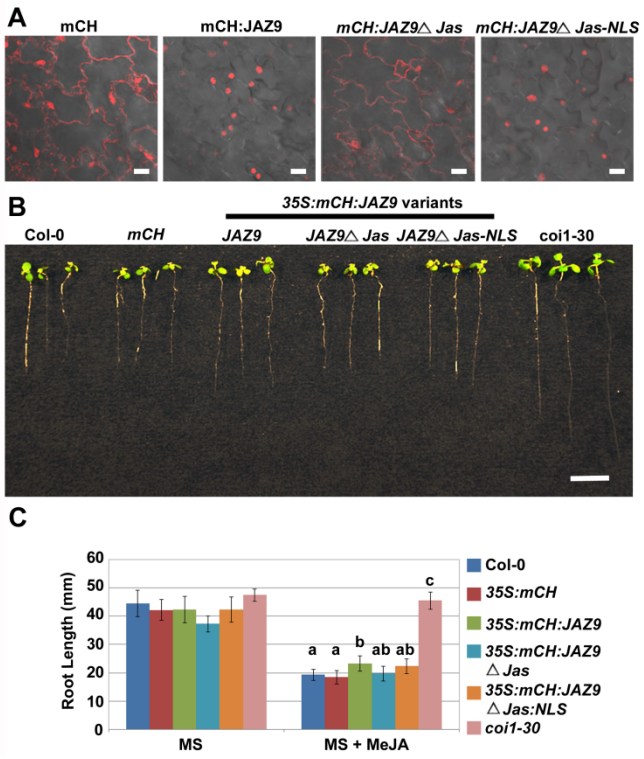
R Jas17 A
KRK Jas16-18 ARA
KRK Jas16-18 AAA

Figure S9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mCH:JAZ9△ Jas
mCH:JAZ9△ Jas-NLS
B
35S:mCH:JAZ9 variants
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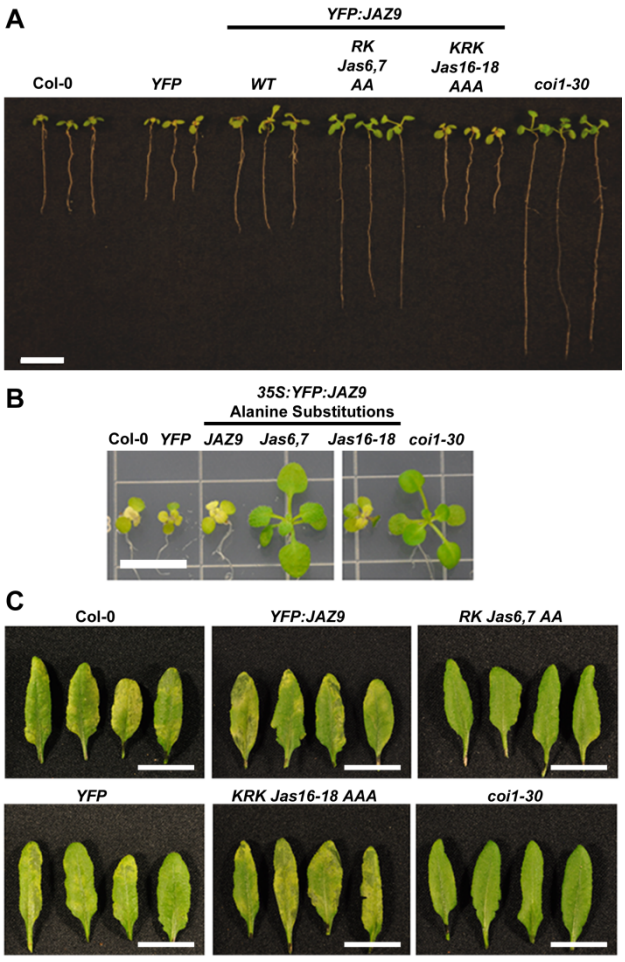
JAZ9
JAZ9△ Jas
JAZ9△ Jas-NLS
coi1-30
C
Root Length (mm)
60
50
40
30
20
10
0
a a b ab ab c
MS
MS + MeJA
Col-0
35S:mCH
35S:mCH:JAZ9
35S:mCH:JAZ9 △Jas
35S:mCH:JAZ9 △Jas:NLS
coi1-30

Figure S10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YFP
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KRK Jas16-18 AAA
coi1-30
B
35S:YFP:JAZ9 Alanine Substitutions
Col-0
YFP
JAZ9
Jas6,7
Jas16-18
coi1-30
C
Col-0
YFP:JAZ9
RK Jas6,7 AA
YFP
KRK Jas16-18 AAA
coi1-30