

Supplementary Materials for

Methylation of a Phosphatase Specifies Dephosphorylation and Degradation of Activated Brassinosteroid Receptors

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Reference

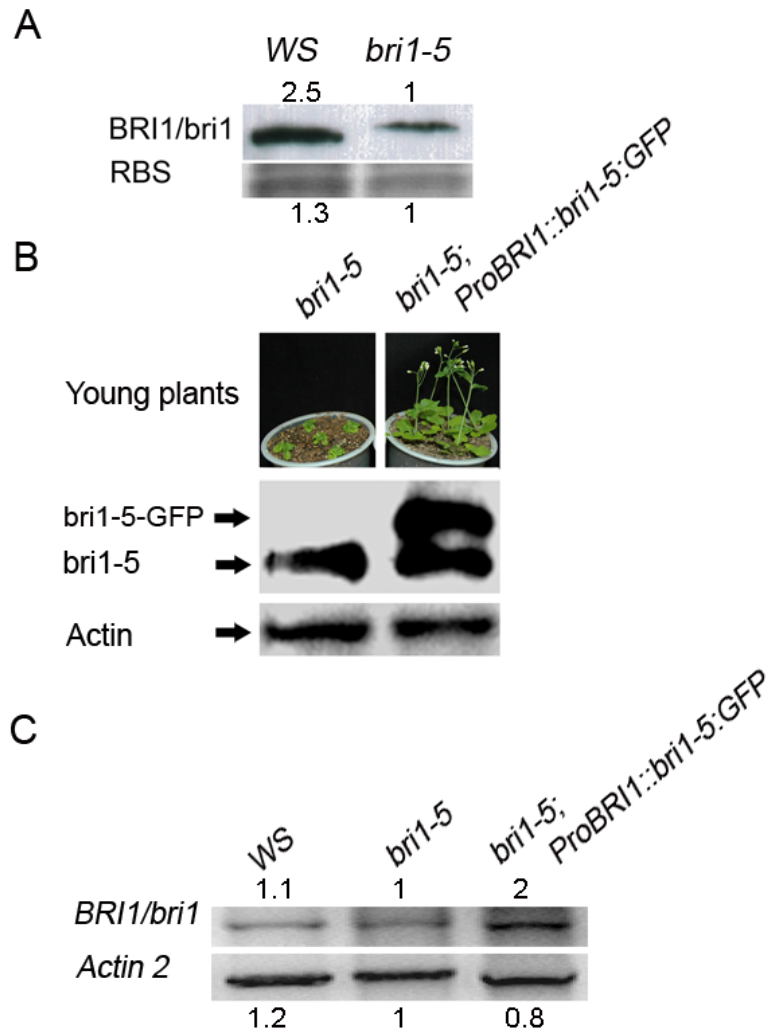


Fig. S1. The abundance of *bri1* was reduced in *bri1-5*, but *bri1-5* phenotypes can be rescued by expression of additional *bri1-5*. **(A)** Western blotting of *bri1* shows that there is less *bri1* in *bri1-5* than there is in wild-type plants (*Ws*). Protein extracts of 14 day-old seedlings. RBS (rubisco), served as the loading control. Relative integrated density was shown above or below each band. Integrated density from the sample of *bri1-5* mutant in each row was set to 1. **(B)** Expression of a *bri1-5* fusion with green fluorescent protein (GFP) under the control of the *BRI1* promoter (*ProBRI1::bri1-5:GFP*) in *bri1-5* largely rescued the growth defects in *bri1-5*. Plants were 4 week-old seedlings. Protein abundance was detected by Western blotting of samples from 14 day-old seedlings. **(C)** The abundance of *BRI1* transcripts was similar in wild type (*Ws*) and *bri1-5* and the abundance of the *bri1-5* (*GFP*) transcript was increased in *ProBRI1::bri1-5:GFP*; *bri1-5* as shown by RT-PCR. *Actin2* (*ACT2*) was used as an internal control. Relative integrated density was shown above or below each band. Integrated density from the sample of *bri1-5* mutant in each row was set to 1. Western blot experiments were repeated 3 times, and RT-PCR experiments 3 times.

bri1 suppressor screen scheme

EMS mutagenized M2 seeds of *bri1-5*



Cold treatment of seeds in the dark at 4C for 4 days



In the light at 22C for 1 day



In the dark at 22C for 4 days



In the light at 22C for 1 day



Transfer seedlings with long hypocotyls
Soil

Fig. S2. Scheme for screening for suppressor of *bri1-5*.

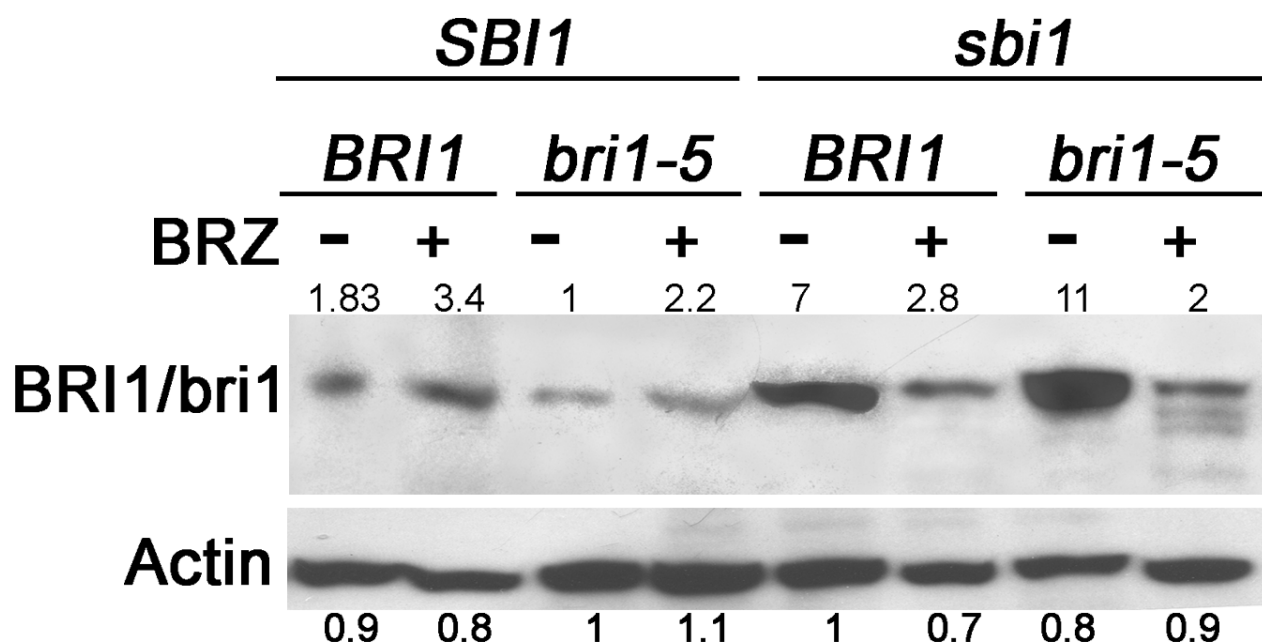


Fig. S3. *sbi1*-dependent *BRI1* or *bri1-5* accumulation requires brassinosteroids. Western blotting of *BRI1* or *bri1* from the extracts of *sbi1*, *bri1-5*, *bri1-5 sbi1* and WT (*Ws*) Seedlings grown in the ½ MS medium with or without 10 μM of the BR biosynthesis brassinazole (BRZ) for 2 weeks. Actin served as the loading control. Inhibition of BR production with BRZ did not reduce the abundance of *BRI1* and *bri1-5* in *SBI1* plants and *bri1-5* mutant plants, whereas the addition of BRZ reduced the abundance of *BRI1* or *bri1-5* in *sbi1* mutant background. Relative integrated density was shown above or below each band. Integrated density from the sample of *bri1-5* mutant in each row was set to 1. Data shown are representative of 3 experiments.

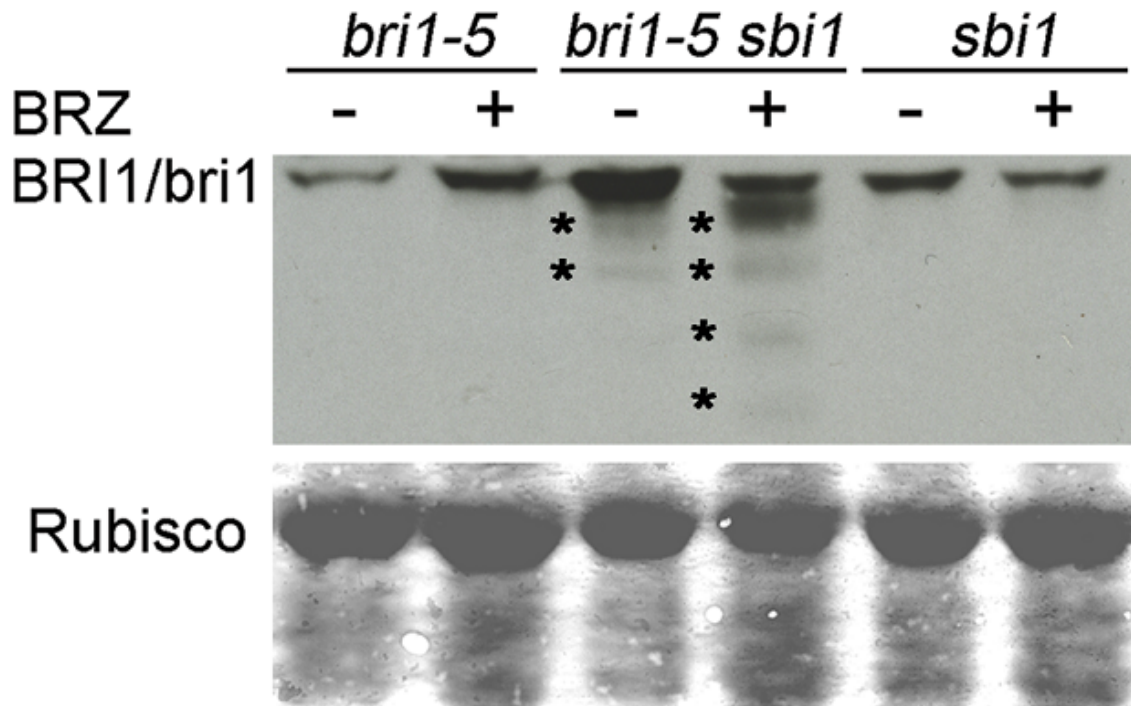


Fig. S4. BRI1 and *bri1-5* in *sbi1* are degraded in the absence of BRs. An extended exposure of Fig. 4D reveals the degradation of *bri1-5* in *bri1-5 sbi1* plants exposed to BRZ. “*” indicates *bri1-5* degradation products. Rubisco (RBS) served as the loading control.

A

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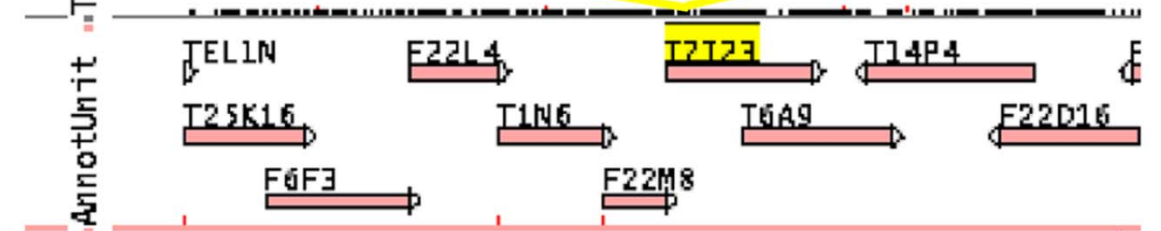
B**C****D**

Fig. S5. Scheme for mapping and cloning of *SBI1*. (A) *SBI1* genomic DNA (*Col*). Capital letters indicate exons. A mutated nucleotide is indicated in a blue colored letter. (B) Gene structure of *SBI1*. Rectangles indicates exons. Red arrow points to the exon with *sbil* mutation. (C) Position of the BAC clone in the north arm of chromosome 1. (D) Chromosome 1.

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AT -----MAESRSNRRAAVQATMDDASASKL 23
Rice -----MDAAAAAAAAAGGGGGGSSVAARSSPASVQATMDDAAASKL 41
DM -----MEPPASAGIAGSTHKFHPCEAVIATMDDASDCKR 35
HS1 -----MATRQRESSITSCCSTSSCDADDEGVRGTCEASLCKR 38
DD MSFSIPPLSSIGSHNNNNKNNNNNNNNNSMNNNSHAPRTSIKSHKESIIGTMDDAASCKL 60
      . . . * : ** : . *

AT SCVKKGYMKDDYVHLFVK-RPVRRSPIINRGYFSRWAAFRKLMSQFLLSGTSS----KKQ 78
Rice SCVNGKGYMKDDYVHFFVR-RTTKRAPIINRGYARWSVLRKLLHQFLGAGNGSMDQNRKQ 100
DM CAVRLGKGYMKDDYIGYFVR-NQERKAPEINRGYFARVKGVMCVEKFLKKTSGN----CQ 89
HS1 FAVSIGYVHDPIYIQHFVRLSKERKAPEINRGYFARVHGVSQLIKAFLRKTECH----CQ 93
DD SAVNVGYYSDFVKYFVK-HPIRRQPLINRGFFSRVEECIEQLVSQLFFFTQYKDIIN---KKQ 116
      . * ** * : : ** : : * ** : : * : * :

AT ILSLGGAGFDTTYFQLLDEG--NGPNLYVELDFKEVTSKKAAVIQNSSQLRDKLG----- 130
Rice ILSLGGAGFDTTFFQLQDEG--IAPYLYVELDFKEVTSKKAAIINHYSQMKEKLG----- 152
DM IINLGCAGFDTLFRLRDTA--HQVKNFIELDFPTVTARKCYTIKRNKALLARIHDED--- 144
HS1 IVNLGAGMDTTFWRLKDED--LLPSKYFEVD FPMIVTRKLHSIKCKPPLSSPILELH--- 148
DD IISLGCAGFDTTYFRLMNNKDIKKDFIYFEVDYDQVISNKIKIIQNHKELQSMIDQEWDSK 176
      * : . ** : * : : * : : : * : : :

AT ----ANASISIDEGQVLSHYKLLPVDLRDIPKLRDVISFADMDLSLPTFIIAECVLIYL 186
Rice ----PEASISIEKGEVRSAHYKLFSDIRDIPKLD SVIQMAEMDPTLPTFIIAECVLIYL 208
DM ----GE--VRLSPTDLHGPSYHLMGVDLRNLDEVD SKLQQAQEVDSYSLPTIFLAECVLVYI 198
HS1 ----SEDTLQMDGHILD SKRYAVIGADLRDLSELEEKLKKCNMNTQLPTLLIAECVLVYM 204
DD YDTNEKLASHVMNHQRVSSKSYRLGSLDNLNLETFK-IFDELEIDYVNVPTLFLSECVLVYI 235
      : : . : . * : * : : . : . : : : ** : : : : : :

AT DPDSSRAIVNWSKTFSTAVFFLYEQIHPDDAFGHQMIRNLESRGCALLSIDASPTLLAK 246
Rice DPASTSSIVIWASDKFSTAIFFLYEQIHPDDAFGEQMIINLESRGCPPLGINATPTLSHK 268
DM EAQNCRNLLKWIQAQKFAAVFVNYEQVMNDRFGDVMLNLRGRGCSLAGVESCLSLDTQ 258
HS1 TPEQSANLLKWAANSFERAMFINYEQVMNDRFGQIMIENLRRRQCDLAGVETCKSLESQ 264
DD PTKCGNDVIQWASNKFSSECFITYEQIKPNDFGRMMIKNIEMKGCPLLSIESFPEIDDQ 295
      . : : * : . * : . * : : . * ** * : * : : * * : : : : :

AT ERLFLDNGWQRAVAWDMLKVYGSFVDTQEKRRIERLELDFEFEEWHMMQEHYCVTYAVND 306
Rice ENLFLDHGWQRAVAWDMKIYND FIDSEERRRERLELDFEFEEWHMMQEHYCVAYGIND 328
DM RNRFKD SGWTGARAWDMVQVYESIS-AAERQRIERLEMLDEGELLQLFQHYCLVAVMLG 317
HS1 KERLLSNGWETASAVDMMELYNRLP-RAEVSRIESLEFLDEMEELLEQLMRHYCLCWATKG 323
DD RKRYNNLGNKTEILD MRHVYSDFINKNRIKETEKLEIFDFEFEEWLIQGHVYVVFAMKS 355
      . . . ** : ** : * : . . * ** : ** * : ** . .

AT AMGIFGDFGFTREGGGERMSSSASSP 332
Rice AKGLFDDFGFKD----- 340
DM VAFQDIDITVEELMSSLNID----- 337
HS1 ----GNEGLKEITY----- 334
DD NSPSILDSYFENSKNK----- 372
      :

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Fig. S6. LCMTs are conserved in eukaryotes. Alignment of SBI1 (LCMT) homologs in other species was performed with CLUSTAL 2.0.10. AT (*Arabidopsis thaliana*, Col), Rice (*Oryza sativa*), DM (*Drosophila melanogaster*), HS1 (*Homo sapiens*, LCMT1), DD (*Dictyostelium discoideum*). Asterisks indicate that identical amino acid residues are identical in the same positions among all homologs; single dots indicate that amino acid residues are identical in the same positions in only some homologs; double dots indicate that biochemically conserved amino acid residues exist in the same positions in different homologs.

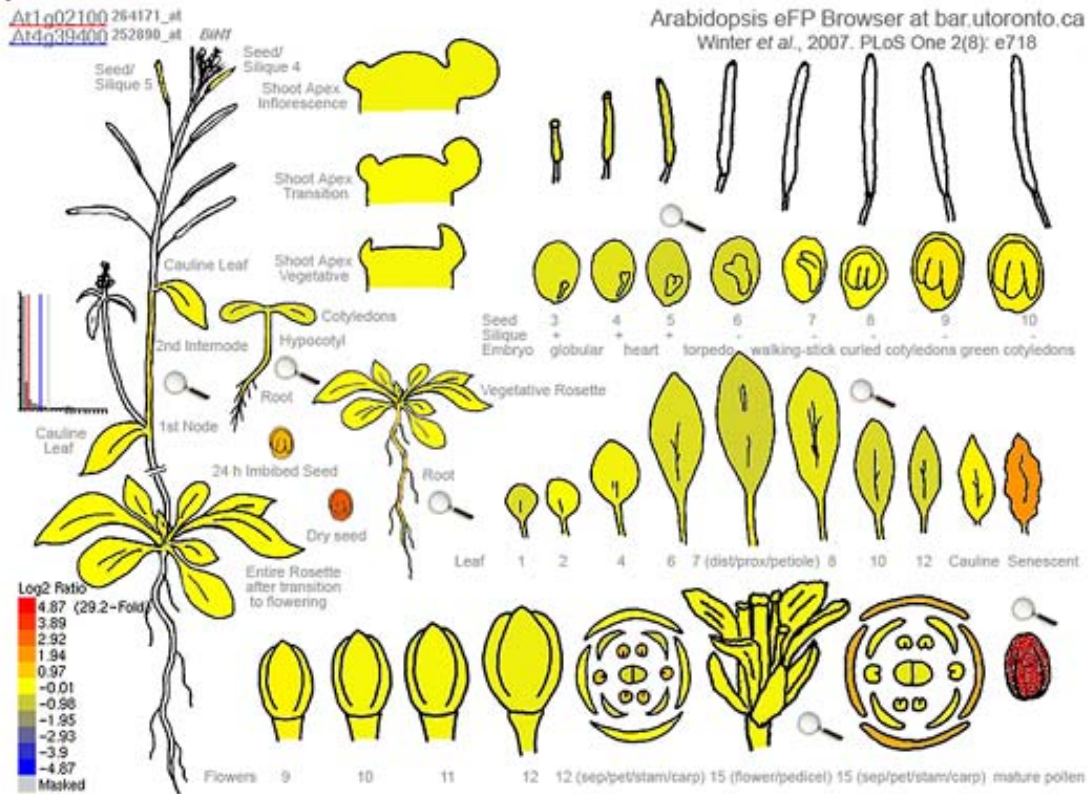
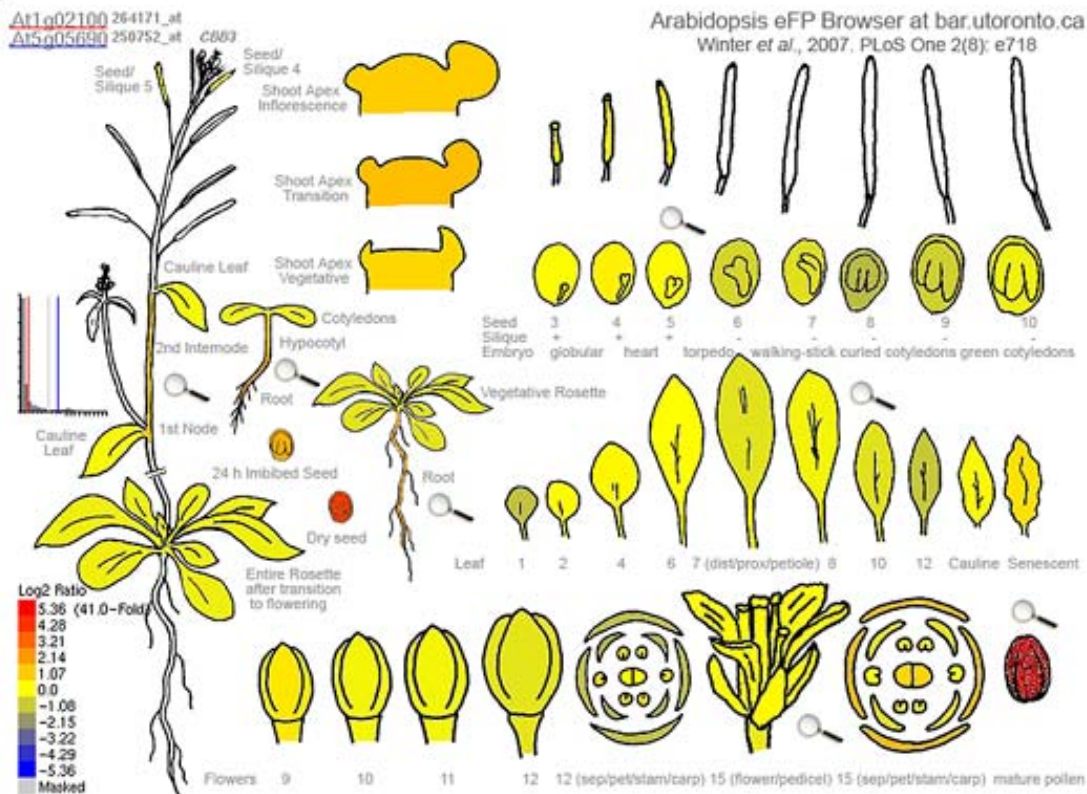
A**B**

Fig. S7. *SBII* (*At1g02100*) is expressed at similar levels as *BR11* (*At4g39400*) and *CPD* (*At5g05690*) in young tissues and expression is higher than these two genes in mature organs. Comparison of tissue-specific expressions between *SBII* and *BR11* (**A**), as well as between *SBII* and *CPD* (**B**). The panel was generated using e-FP browser from (<http://bbc.botany.utoronto.ca/efp/cgi-bin/efpWeb.cgi>) (1). The relative expression of *SBII* compared to *BR11* or to *CPD* in various organs is shown by a color scale: red color indicate higher *SBII* expression, blue indicates higher *BR11* or *CPD* expression, and yellow suggests similar expression level of *SBII* and *BR11* or *CPD*. Images can be regenerated with respective loci on (<http://bbc.botany.utoronto.ca/efp/cgi-bin/efpWeb.cgi>).

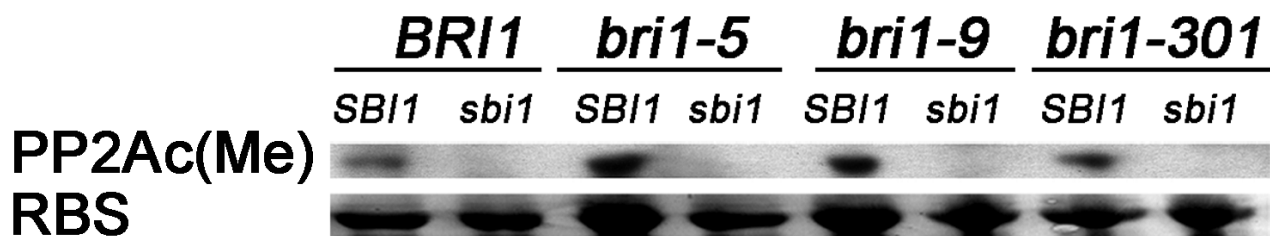


Fig. S9. Methylated PP2Ac were undetectable in the *sbi1* mutant background. Western blotting shows the absence of methylated PP2Ac [PP2Ac(Me)] in the indicated *sbi1* mutant and different *sbi1 bri1* double mutants. Rubisco (RBS) was the loading control. Extracts were prepared from 14-day-old seedlings. Data shown are representative of 3 experiments.

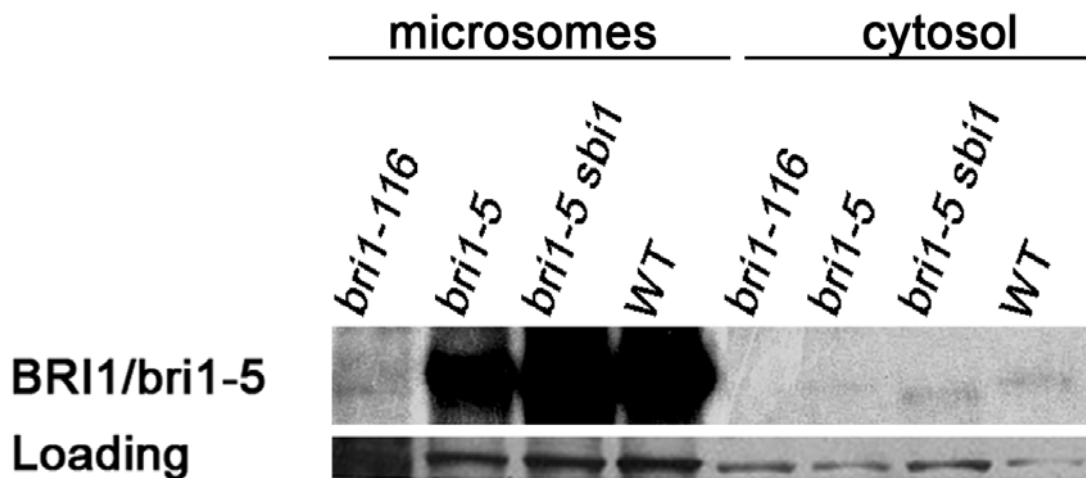


Fig. S10. Distribution of BRI1 or *bri1* in subcellular fractions. Samples in Fig. 6C were tested for quality of microsomal and cytosolic fractions on the basis of the relative amount of membrane-localized BRI1 or *bri1* in the membranous and cytosolic fractions. Nonspecific bands served as the loading control. *bri1-116* is a *bri1* null allele.

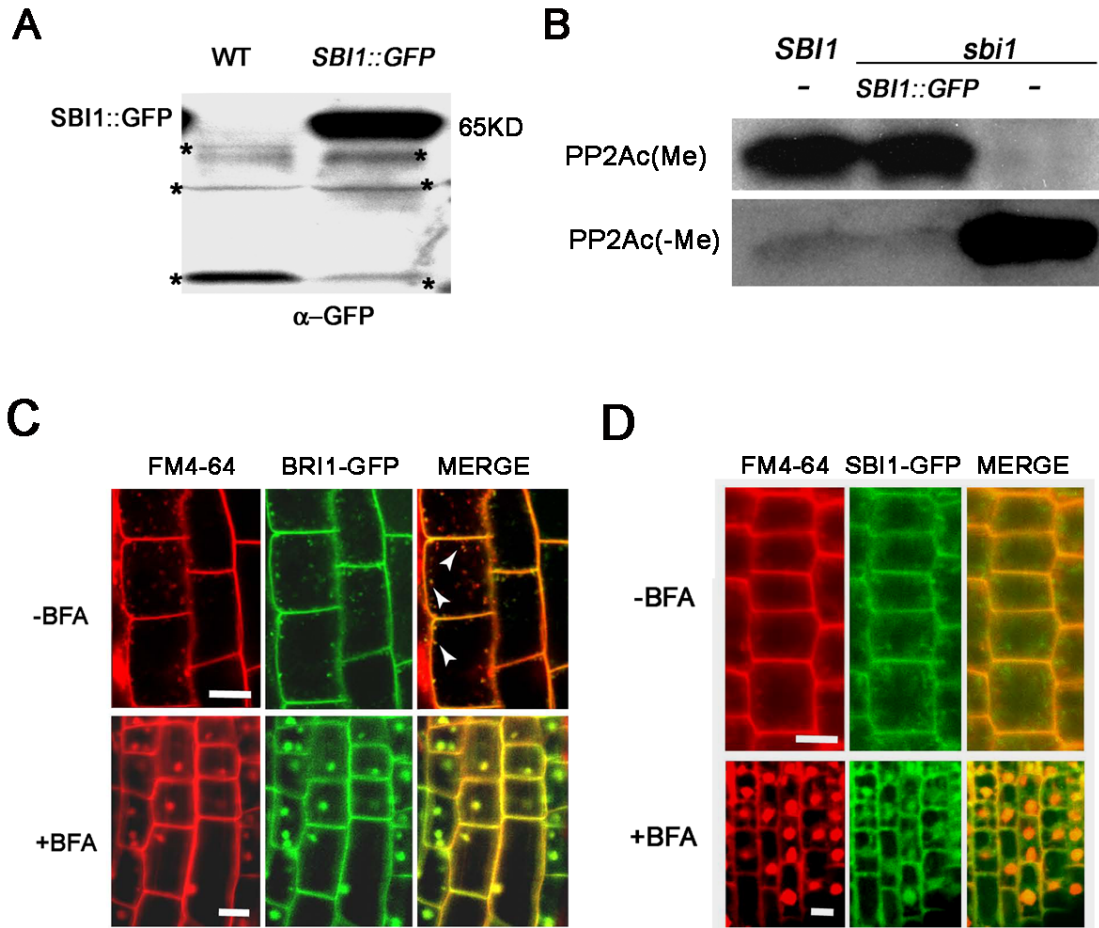


Fig. S11. SBI1 partially localizes to membranous compartments. **(A)** Full-length SBI1::GFP was detected by antibodies that recognize GFP (α -GFP). **(B)** Expression of 35S::SBI1::GFP in the *sbi1* mutant rescued the loss of LCMT activity toward PP2Ac in *sbi1*. Methylated PP2Ac [PP2Ac(me)] and unmethylated PP2Ac [PP2Ac(-me)] were detected with antibodies against peptides of PP2Ac [PP2Ac(me)] and unmethylated PP2Ac [PP2Ac(-me)] respectively. **(C)** BRI1-GFP (green) colocalized with FM4-64 (red) at the plasma membrane and endosomes of *Arabidopsis* root tips. Upper panel, images of BRI1-GFP and FM4-64. Lower panel, images of BRI1-GFP and FM4-64 in tissues treated with brefeldin A (BFA). Arrowheads indicate putative endosomes. Scale bars: 10 μ m. **(D)** SBI1-GFP (green) partially colocalized with FM4-64 (red) at the plasma membrane and putative endosomes *Arabidopsis* root tips. SBI1 was also detected in the cytoplasm. Upper panels, images of SBI1-GFP and FM4-64. Lower panels, images of SBI1-GFP and FM4-64 in tissues treated with BFA. Arrowheads indicate putative endosomes. Scale bars: 10 μ m.

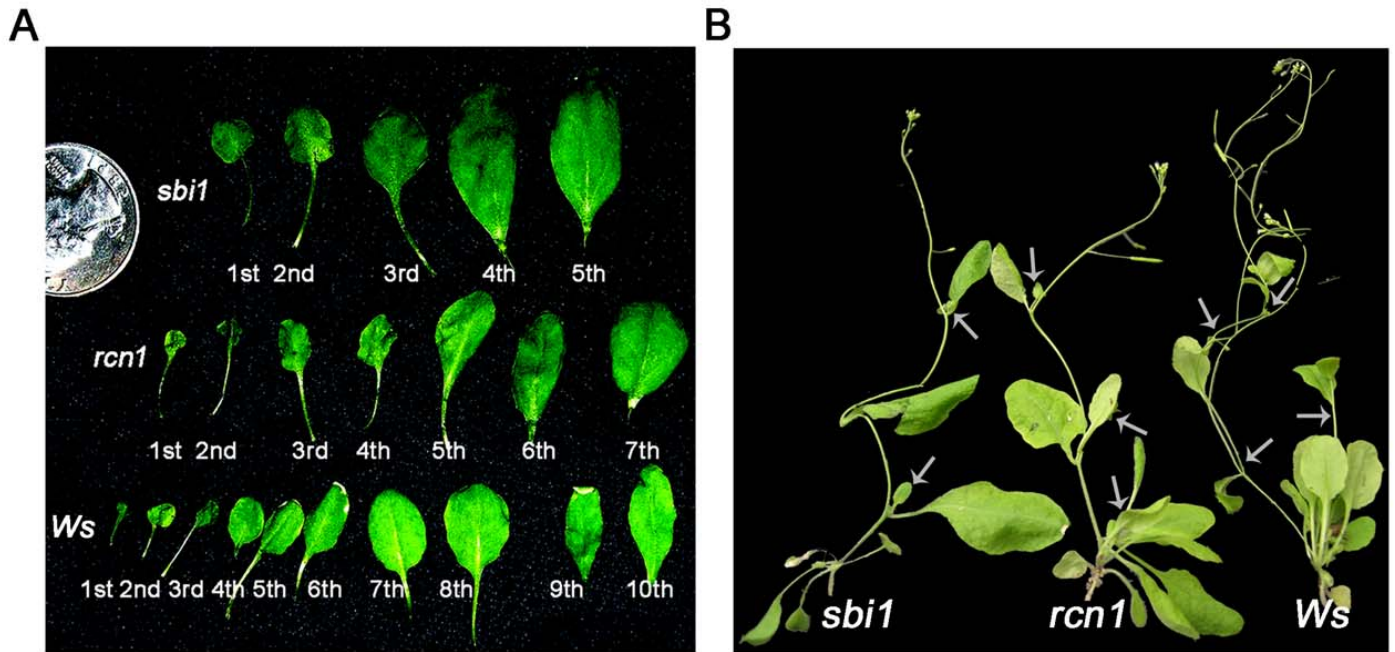


Fig. S12. *rcn1* and *sbi1* mutants have similar phenotypes. **(A)** Fewer leaves were produced by 5-week-old *rcn1* (*pp2aa1*) and *sbi1* plants than were produced by wild-type (*Ws*) plants. **(B)** 5-week-old *sbi1* and *rcn1* plants exhibit a higher degree of apical dominance than is seen in WT (*Ws*) plants. Only two small axillary buds were present in the *sbi1* plant and three axillary buds were present in the *rcn1* plant; whereas three branches and one axillary bud were present in the wild-type plant (arrowheads point to axillary buds or branches).

A

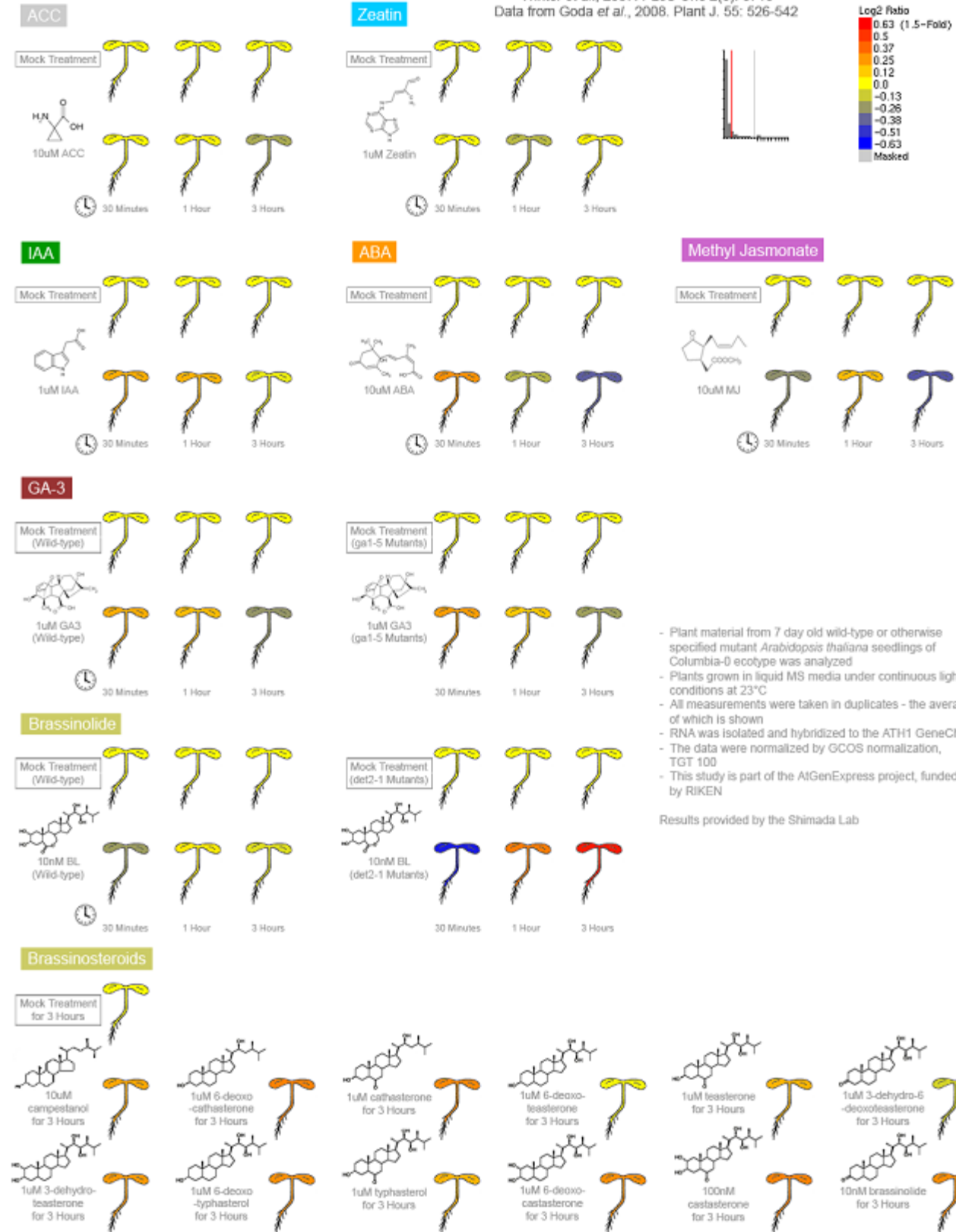
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☐ = Control

Arabidopsis eFP Browser at bar.utoronto.ca

Winter et al., 2007. PLoS One 2(8): e718

Data from Goda et al., 2008. Plant J. 55: 526-542



B

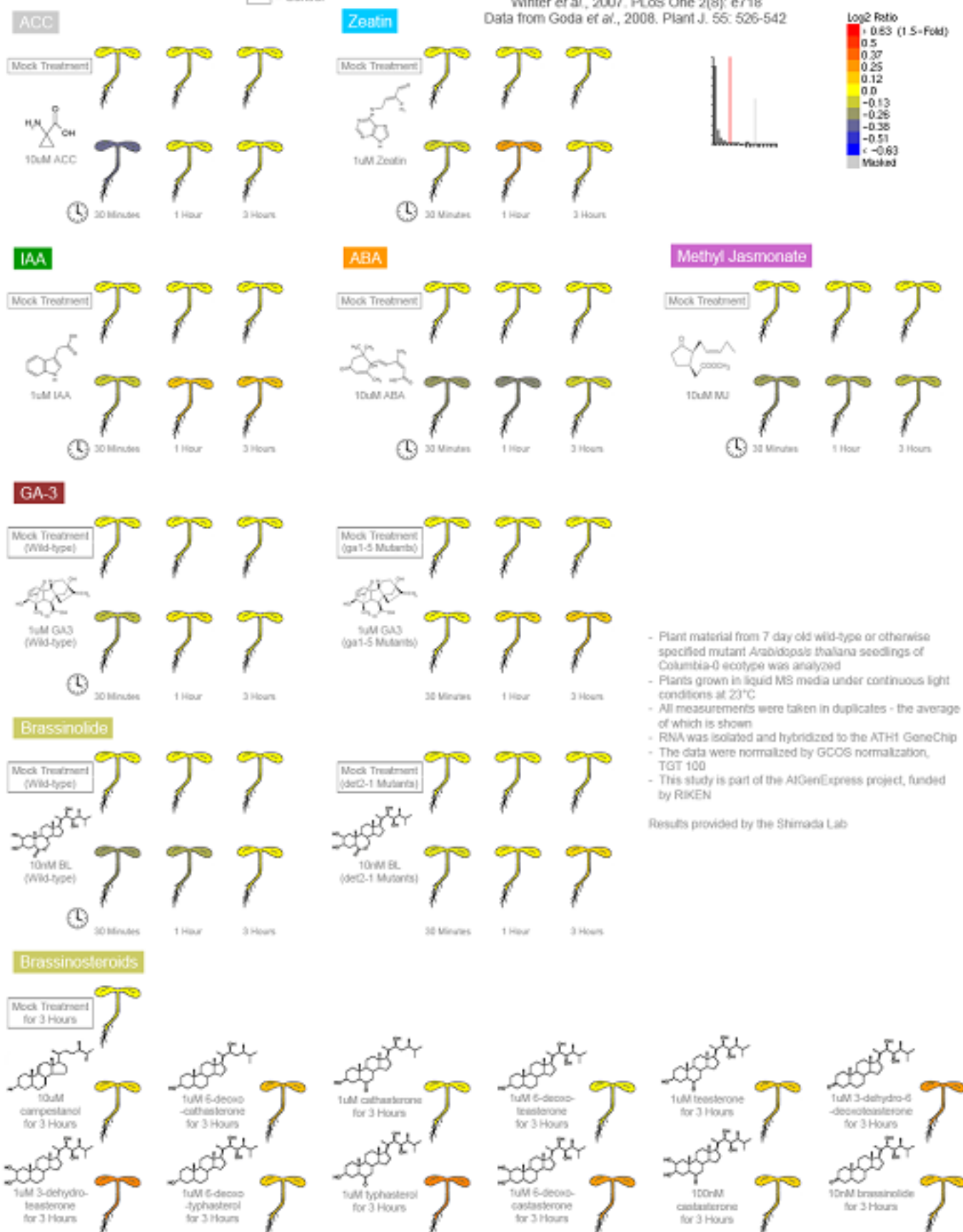
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Data from Goda et al., 2008, Plant J. 55: 526-542



- Plant material from 7 day old wild-type or otherwise specified mutant *Arabidopsis thaliana* seedlings of Columbia-0 ecotype was analyzed
- Plants grown in liquid MS media under continuous light conditions at 23°C
- All measurements were taken in duplicates - the average of which is shown
- RNA was isolated and hybridized to the ATH1 GeneChip
- The data were normalized by GCOS normalization, TGT 100
- This study is part of the AGenExpress project, funded by RIKEN

Results provided by the Shimada Lab

C

At2g26710 267614_at CYP72B1

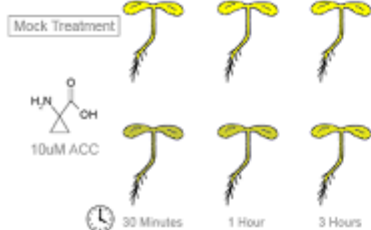
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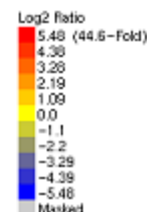
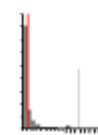
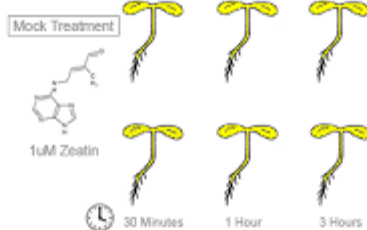
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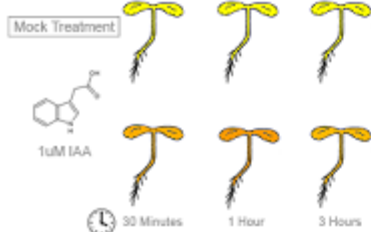
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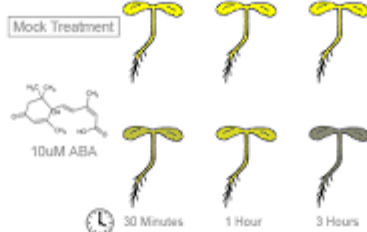
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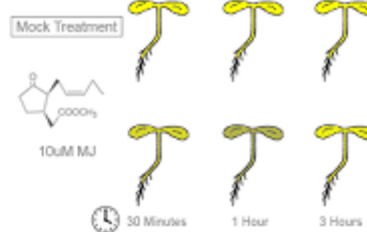
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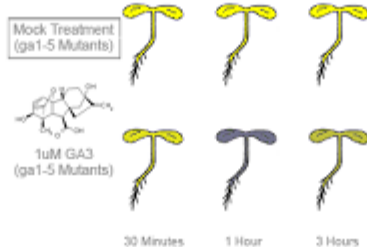
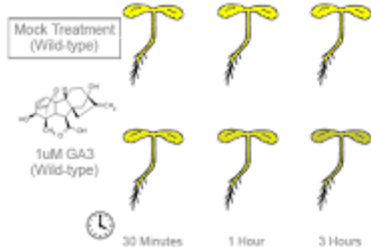
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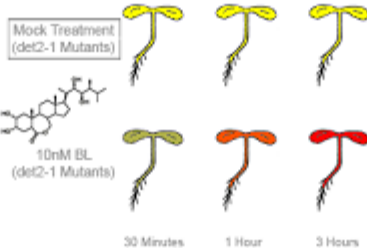
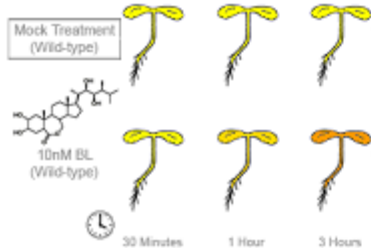
Methyl Jasmonate



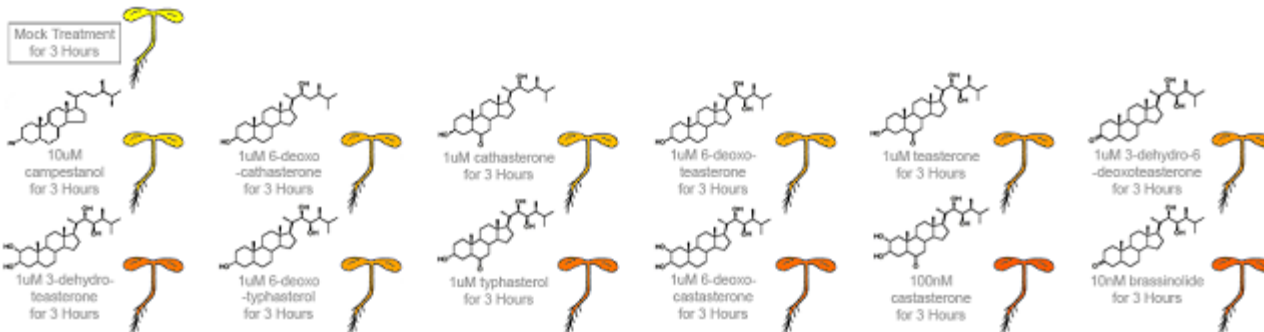
GA-3



Brassinolide



Brassinosteroids



- Plant material from 7 day old wild-type or otherwise specified mutant *Arabidopsis thaliana* seedlings of Columbia-0 ecotype was analyzed
- Plants grown in liquid MS media under continuous light conditions at 23°C
- All measurements were taken in duplicates - the average of which is shown
- RNA was isolated and hybridized to the ATH1 GeneChip
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- This study is part of the AtGenExpress project, funded by RIKEN

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D

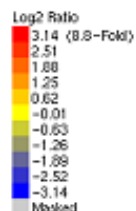
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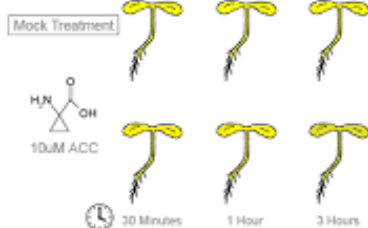
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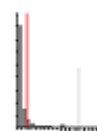
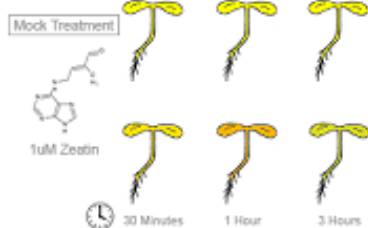
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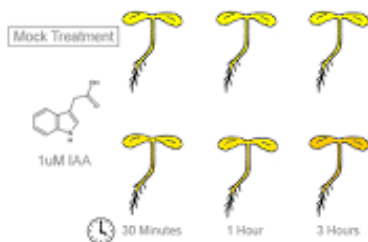
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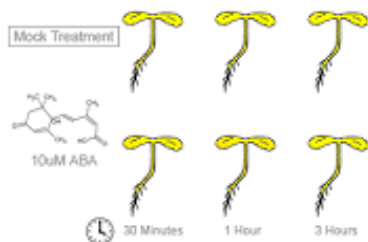
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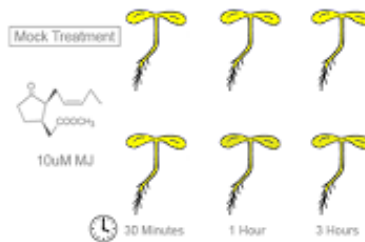
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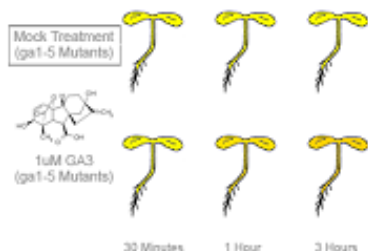
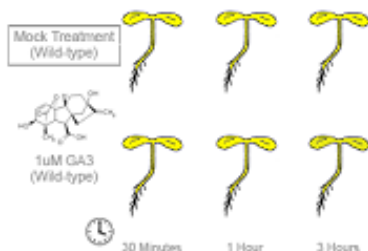
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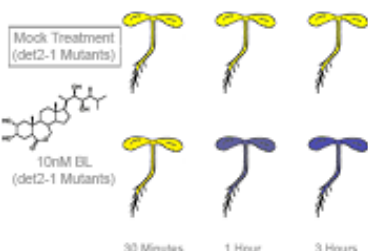
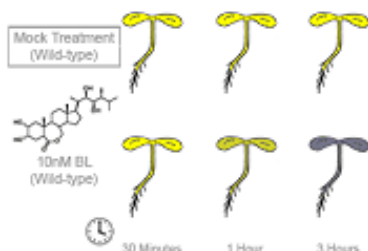
Methyl Jasmonate



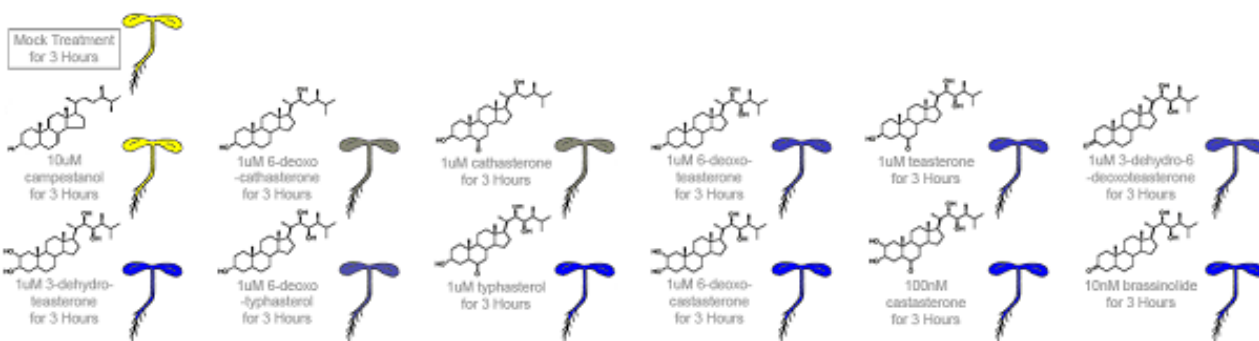
GA-3



Brassinolide



Brassinosteroids



- Plant material from 7 day old wild-type or otherwise specified mutant *Arabidopsis thaliana* seedlings of Columbia-0 ecotype was analyzed
- Plants grown in liquid MS media under continuous light conditions at 23°C
- All measurements were taken in duplicates - the average of which is shown
- RNA was isolated and hybridized to the ATH1 GeneChip
- The data were normalized by GCOS normalization, TGT 100
- This study is part of the AtGenExpress project, funded by RIKEN

Results provided by the Shimada Lab

E

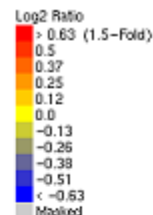
At3g18780 257749_at DER1

□ = Control

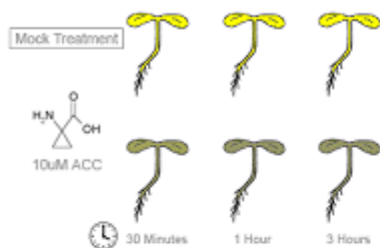
Arabidopsis eFP Browser at bar.utoronto.ca

Winter et al., 2007. PLoS One 2(8): e718

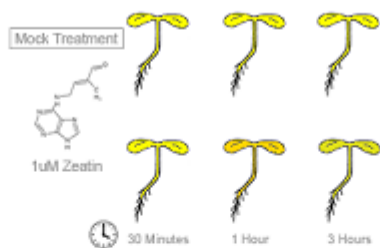
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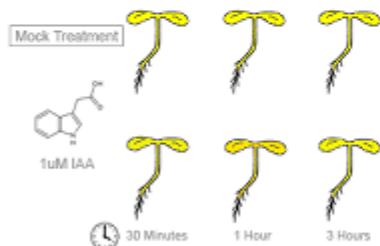
ACC



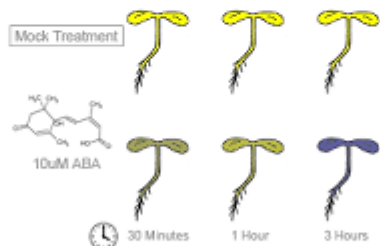
Zeatin



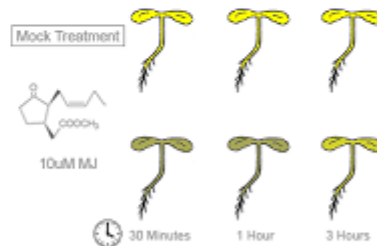
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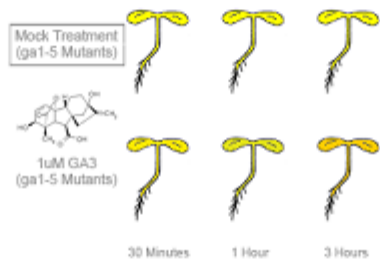
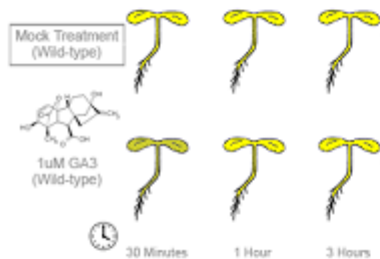
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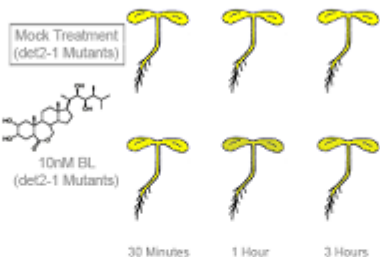
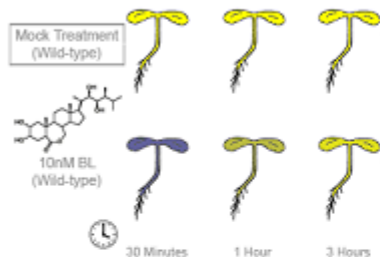
Methyl Jasmonate



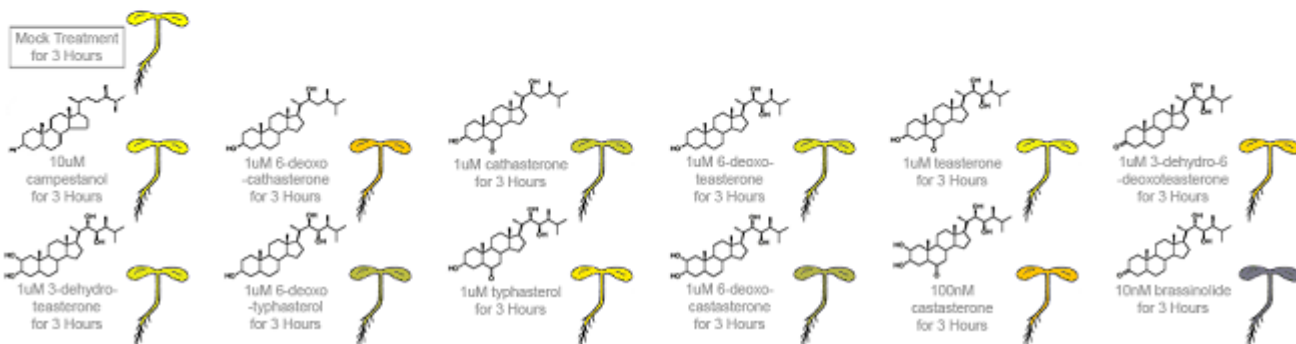
GA-3



Brassinolide



Brassinosteroids



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Results provided by the Shimada Lab

Fig. S13. Expression of *SBII* was specifically increased by BRs. All the panels were generated with the relative gene expression levels using e-FP browser from (<http://bbc.botany.utoronto.ca/efp/cgi-bin/efpWeb.cgi>) (1). All seedlings used for the different hormone treatments were wild type (WT, *Col.*) except for the second and third sets of the experiments with brassinosteroid treatments, in which the seedlings of *det2* mutants were used. Details of the experimental design can be obtained through the website (<http://affymetrix.arabidopsis.info/narrays/experimentpage.pl?experimentid=176>). Red color bars indicate that the gene expression was high; the blue bars indicate that the gene expression was low. **(A)** *SBII* (*At1g02100*) was specifically increased when wild-type (WT) or the BR biosynthesis mutant *det2* was treated with BRs but not any other hormones. **(B)** No clear expression pattern of *RCN1* (*At1g25490*) was found in WT or *det2* mutants treated with BRs. **(C)** Expression of the BR inactivation gene *BASI* (*At2g26710*) was increased in WT and *det2* mutants by treatment with BRs. **(D)** The expression of the BR biosynthesis gene *DWF4* (*At3g50660*) was decreased in WT and *det2* by treatment with BRs. **(E)** The expression of actin *ACT2* (*At3g18780*) was not affected by any of the hormone treatments. ACC, 1-Aminocyclopropane-1-carboxylic acid (ACC); zeatin, a member of cytokinins; IAA, indole acetic acid; ABA, abscisic acid; GA-3, gibberellin-3. Images can be regenerated with respective loci on (<http://bbc.botany.utoronto.ca/efp/cgi-bin/efpWeb.cgi>).

Reference

1. D. Winter *et al.*, An "electronic fluorescent pictograph" browser for exploring and analyzing large-scale biological data sets. *PLoS One* **2**, e718 (2007).