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Supplemental Information

ROPGAP-dependent interaction between brassinosteroid and ROP2-GTPase signaling controls pavement cell shape in *Arabidopsis*

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Figure S1 Complementation of *phgap1phgap2* mutant. Related to Figure 2.

(A) Abaxial cotyledon epidermis of *phgap1phgap2* plants expressing *PHGAP1_{pro}:GFP-gPHGAP1* and *PHGAP2_{pro}:GFP-gPHGAP2* at different transcript levels at 5 days post germination (dpg). The variation in cell circularity is shown with a color code. Scale bar, 100 μm .

(B) Expression of *PHGAP1* and *PHGAP2* in *PHGAP1_{pro}:GFP-gPHGAP1/phgap1phgap2* and *PHGAP2_{pro}:GFP-gPHGAP2/phgap1phgap2*, respectively, at 5 dpg measured by qRT-PCR. Three biological replicates were analyzed and three technical repeats were done per sample. *ACTIN2* was used as reference gene and transcript levels were normalized to the endogenous expression in the wild type (Col-0). Bars represent SD.

(C and D) Quantification of pavement cell circularity (C) and lobe counts (D) in *phgap1phgap2* mutant plants complemented with either *PHGAP1_{pro}:GFP-gPHGAP1* (top) or *PHGAP2_{pro}:GFP-gPHGAP2* (bottom). Cells $\geq 1400 \mu\text{m}^2$ from 5-6 individual cotyledons were segmented and measured with the 'PaCeQuant' Image J plugin. *n*, number of cells analyzed. Data are shown as individual value plots in columns and with whiskers representing means and SD. Significant differences were determined with single-factor ANOVA compared to Col-0. *** $P < 0.001$. ns, no significant difference.

(E) Fluorescence intensity images of GFP-PHGAP1 and GFP-PHGAP2 in abaxial cotyledon epidermis of plants at 5 dpg described in (A). 3D reconstruction was generated to show the anticlinal face of the cells. Scale bars, 20 μm .

Figure S2 Expression patterns of GFP-PHGAP1, GFP-PHGAP2 and GFP-ROP2. Related to Figure 2 and Figure 4.

(A) Expression of GFP-PHGAP1 (*PHGAP1_{pro}:GFP-gPHGAP1/phgap1phgap2*, line #3.2.3) and GFP-PHGAP2 (*PHGAP2_{pro}:GFP-gPHGAP2/phgap1phgap2*, line #3.1.1) shown as fluorescence intensity in the abaxial cotyledon epidermis of *Arabidopsis* at 2, 3, 5 and 7 days post germination (dpg). Scale bars, 50 μ m.

(B) Expression of the *NLSGFP* promoter reporters for *PHGAP1* and *PHGAP2* in the abaxial cotyledon epidermis at 5 dpg. Before imaging, cotyledons were stained with 50 μ M FM4-64. Scale bar, 50 μ m.

(C) Expression of GFP-ROP2 (*ROP2_{pro}:GFP-ROP2/rop2rop6*) shown as fluorescence intensity in the abaxial cotyledon epidermis at 5 dpg. Scale bar, 50 μ m.

Figure S3 Exogenous BL and BIK affect PHGAP2 turnover and activate ROP6. Related to Figure 4.

(A) Inhibition of BIN2 kinase activity promoted the GFP-PHGAP2 degradation in *PHGAP2_{pro}:GFP-gPHGAP2/phgap1phgap2* (#7.8.1) seedlings at 5 days post germination (dpg) grown on agar plates and treated with liquid medium containing 100 nM brassinolide (BL) or 50 μ M bikinin (BIK) for 1, 3, 6 and 12 h in the presence of 100 μ M cycloheximide (CHX). The GFP-PHGAP2 protein was detected on western blot (WB) with the α -GFP antibody. Tubulin (Tub), detected with α -Tub antibody, was used as a loading control. Numbers below the blot indicate the relative band intensities of the GFP/Tub normalized to 0 h (bottom), $\pm$ SD (middle) and *P*-value from a Student's *t*-test compared to 0 h (top). 4 biological replicates were performed (*n*=4).

(B) ROP6 activated by exogenous brassinolide (BL) and bikinin (BIK) in wild type and *phgap1phgap2* seedlings. Protoplasts from 4-week-old wild type (Col-0) and *phgap1phgap2* leaves were treated with different concentrations of BL or BIK for 10 min. GTP-bound active ROP6 was pulled down with GST-RIC1-conjugated beads and detected by western blot with anti-ROP6 antibodies. Numbers below the blot show the relative band intensities of active ROP6/ non-specific band normalized with 0 h (bottom), $\pm$ SD (middle) and *P*-value from a Student's *t*-test compared to 0 h (top). 3 biological replicates were performed (*n*=3).

Figure S4 Phenotypes of the *AtSK* mutants. Related to Figure 6.

(A) Seedling phenotypes of wild type in Wassilewskija (WS) background, *atsk^{quad}* and *atsk^{sext}* mutants at 5 days post germination (dpg) in the T1 generation.

(B) RT-qPCR of *AtSK11*, *AtSK12* and *AtSK13* expression in WS and different independent transgenic seedlings of the *atsk^{sext}* mutant at 5 dpg. The qRT-PCR was done once with three technical repeats per sample. *ACTIN2* was used as reference gene and transcript levels were normalized to the endogenous expression in the wild type (WS). Bars represent SD.

(C) Quantification of epidermal pavement cell circularity and lobe counts in seedlings of WS and *atsk^{sext}* mutant at 5 dpg. Cells $\geq 1400 \mu\text{m}^2$ from 5-6 individual cotyledons were segmented and measured with the 'PaCeQuant' Image J plugin. *n*, number of cells analyzed. Data are shown as individual value plots in columns and with whiskers representing means and SD. Significant differences were determined with single-factor ANOVA compared to the wild type (WS). * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$. ns, no significant difference.

Figure S5 Generation of the *atsk^{octu}* mutant by CRISPR. Related to Figure 6.

(A) Phenotypes of T1 seedlings of *atsk^{octu}* and *GFP-PHGAP2/atsk^{octu}* at 5 days post germination (dpg). Col-0 and *PHGAP2_{pro}:GFP-gPHGAP2/phgap1phgap2* (line #3.1.1) were used as controls.

(B) Gene editing efficiency analysis in plants shown in (A) (underlined). Genomic DNA isolated from T1 seedlings at 5 dpg were sequenced with the primers listed in Table S1. The sequencing results were analyzed with the online software ICE (<https://ice.synthego.com/#/>). Detailed analyses are shown in Data S2. InDel % indicates the percentage of the cell population with insertions or deletions. Knockout score represents the proportion of cells that have either a frameshift-inducing InDel or a large InDel in a protein-coding region with a length of ≥ 21 bp to possibly generate a complete loss-of-function mutation.

(C) Abaxial cotyledon epidermis of different T1 *atsk^{octu}* mutants shown in (A) at 5 dpg. The variation in cell circularity is indicated with a color code. Scale bar, 100 μ m.

(D) Localization of GFP-PHGAP2 shown as fluorescence intensity in the abaxial cotyledon epidermis of *phgap1phgap2/atsk^{octu}* plants in the T1 generation at 5 dpg. Scale bar, 50 μ m.

(E) Quantification of the images in (D). Undulations were classified into peak, partial peak and diffuse based on their relative indentation enrichment (RIE) value of GFP-PHGAP2.

Figure S6 Epidermis-specific CRISPR. Related to Figure 6.

The *ML1* promoter driving *Cas9* can efficiently knock out GFP in cotyledon epidermal cells. The *ML1_{pro}:Cas9-P2A-mCherry* expression cassette combined with a gRNA targeting the GFP-coding sequence (*ML1_{pro}:Cas9-P2A-mCherry;GFP-1*) was transformed into Col-0 plants expressing the nuclear localized GFP-GUS form a ubiquitous *HISTONE THREE-RELATED5* (*HTR5*) promoter. Cotyledons were stained with propidium iodide (PI) (50 µg/mL) before imaging. Scale bar, 50 µm.

Primer name	Primer sequence	Purpose
Cloning		
Pro-PHGAP1_attB4f	GGGGACAACCTTTGTATAGAAAAGTTGGGatgttcattaacacacgetg	PHGAP1 promoter cloning
Pro-PHGAP1_attB1r	GGGGACTGCTTTTTTTGTACAAACTTGTgcagaagtacacactaat	PHGAP1 promoter cloning
gPHGAP1_attB2r	GGGGACAGCTTTCTTGTACAAAGTGGGGATGGAGGCTTCTCTAGCTGT	PHGAP1 gDNA cloning
gPHGAP1_attB3	GGGGACAACCTTTGTATAATAAAAGTTGTCTAATTCCATGGTGGAGAAG	PHGAP1 gDNA cloning
PHGAP1-B1	GGGGACAAGTTTGTACAAAAAAGCAGGCTTAATGGAGGCTTCTCTAGCTGT	PHGAP1 cDNA cloning
PHGAP1-B3	GGGGACAACCTTTGTATAATAAAAGTTGGAATGGAGGCTTCTCTAGCTGT	PHGAP1 BiFC entry clone
PHGAP1-B2	GGGGACCACTTTGTACAAGAAAGCTGGGTGCTAATTCCATGTGGGAGAAG	PHGAP1 BiFC entry clone
PHGAP1 (1-380) attB2st	GGGGACCACTTTGTACAAGAAAGCTGGGTGCTAGGAGCATCTCCCAAGACCTT	Truncated PHGAP1 (1-380) cloning
Pro-PHGAP2_attB4f_long	GGGGACAACCTTTGTATAGAAAAGTTGGGcagcgccgctggcgccg	PHGAP2 promoter cloning
Pro-PHGAP2_attB1r	GGGGACTGCTTTTTTTGTACAAACTTGTgcctcaacagcataatctc	PHGAP2 promoter cloning
gPHGAP2_attB2r	GGGGACAGCTTTCTTGTACAAAGTGGGGATGGAGGCTTCTTTAGCGGC	PHGAP2 gDNA cloning
gPHGAP2_attB3	GGGGACAACCTTTGTATAATAAAAGTTGTTTAGTTCCAAGGTGGAGATG	PHGAP2 gDNA cloning
PHGAP2-B1	GGGGACAAGTTTGTACAAAAAAGCAGGCTTAATGGAGGCTTCTTTAGCGGC	PHGAP2 BiFC entry clone
PHGAP2-B2	GGGGACCACTTTGTACAAGAAAGCTGGGTGTTAGTTCCAAGGTGGAGATG	PHGAP2 cDNA cloning
PHGAP2-B4	GGGGACAACCTTTGTATAGAAAAGTTGGGTGTTAGTTCCAAGGTGGAGATG	PHGAP2 BiFC entry clone
PHGAP2(1-420)attB2st	GGGGACCACTTTGTACAAGAAAGCTGGGTGTTATGCACGGTCATTATCGTCATC	Truncated PHGAP2 (1-420) cloning
PHGAP2-allA_attB2r	GGGGACAGCTTTCTTGTACAAAGTGGGGATGGAGGCTGCTTTAGCGGC	PHGAP2(19A) gDNA cloning
PHGAP2-allA_attB3	GGGGACAACCTTTGTATAATAAAAGTTGTTTAGTTCCAAGGTGGAGCTG	PHGAP2(19A) gDNA cloning
BIN2-B1	GGGGACAAGTTTGTACAAAAAAGCAGGCTTAATGGCTGATGATAAGGAGAT	BIN2 BiFC entry clone
BIN2-B4	GGGGACAACCTTTGTATAGAAAAGTTGGGTGAGTTCCAGATTGATTCAAGA	BIN2 BiFC entry clone
BIN2-B3	GGGGACAACCTTTGTATAATAAAAGTTGGAATGGCTGATGATAAGGAGAT	BIN2 BiFC entry clone
BIN2-B2	GGGGACCACTTTGTACAAGAAAGCTGGGTGAGTTCCAGATTGATTCAAGA	BIN2 BiFC entry clone
ROP2-attB3	GGGGACAACCTTTGTATAATAAAAGTTGTAATGGCGTCAAGGTTTATAAAGTGT	ROP2 BiFC entry clone
ROP2_attb2	GGGGACCACTTTGTACAAGAAAGCTGGGTGTCACAAGAACGCGCAACGGT	ROP2 BiFC entry clone
KIB1_AttB1F	GGGGACAAGTTTGTACAAAAAAGCAGGCTTATGACACATAAGAAGCAGAAG	KIB1 cDNA cloning
KIB1_AttB3	GGGGACAACCTTTGTATAATAAAAGTTGTAATGACACATAAGAAGCAGAAGA	KIB1 BiFC entry clone
KIB1_AttB2_stop	GGGGACCACTTTGTACAAGAAAGCTGGGTATTAAGCAACCATTGGAAG	KIB1 cDNA cloning
KIB1_AttB2_nostop	GGGGACCACTTTGTACAAGAAAGCTGGGTAAAGCAACCATTGGAAG	KIB1 BiFC entry clone
pML1_Fw_A	AGAAGTGAAGCTTGGTCTCAACCTagaaaaacaagaacaaaac	ML1 promoter cloning
pML1_Rv_B	AGGGCGAGAATTCGGTCTCATGTTgatgatgatgatgcctatc	ML1 promoter cloning
CRISPR gRNAs		
AtSK11_gRNA1_Fw	TTTTGAAGACATATTGACCGTGAGCTTCAAACCATGGTTTTAGAGCTAGAAATAGC	Guide RNA of AtSK11
AtSK11_gRNA2_Rv	TTTTGAAGACTTAAACCTGAAAAAGATGAGCTTTACCAATCACTACTTCGACTC	Guide RNA of AtSK11
AtSK12_gRNA1_Fw	TTTTGAAGACATATTGGGGCATAGAGCCTAGTGCCGGTTTTAGAGCTAGAAATAGC	Guide RNA of AtSK12
AtSK12_gRNA2_Rv	TTTTGAAGACTTAAACCGGGTAATCTATCAGCATCACAATCACTACTTCGACTC	Guide RNA of AtSK12

AtSK13_gRNA1_Fw	TTTTGAAGACATATTGACTATAGTCGGGCAAACCAGGTTTTA GAGCTAGAAATAGC	Guide RNA of AtSK13
AtSK13_gRNA2_Rv	TTTTGAAGACTTAAACCCATGAATATAAGCCAAAGCCAATCA CTACTTCGACTC	Guide RNA of AtSK13
AtSK21_gRNA1_Fw	TTTTGAAGACATATTGTTTGGAGACTGGAGAAACCGGTTTTA GAGCTAGAAATAGC	Guide RNA of AtSK21
AtSK21_gRNA2_Rv	TTTTGAAGACTTAAACCTTGTCTATGTTAACTTTACAATCAC TACTTCGACTC	Guide RNA of AtSK21
AtSK22_gRNA1_Fw	TTTTGAAGACATATTGACTATACTAGTTCTAATCAGGTTTTA GAGCTAGAAATAGC	Guide RNA of AtSK22
AtSK22_gRNA2_Rv	TTTTGAAGACTTAAACCTACTATTGGAGGCAAAAACCAATCA CTACTTCGACTC	Guide RNA of AtSK22
AtSK23_gRNA1_Fw	TTTTGAAGACATATTGGCTCTTCCTCAATCTCGTTAGTTTTAG AGCTAGAAATAGC	Guide RNA of AtSK23
AtSK23_gRNA2_Rv	TTTTGAAGACTTAAACCTGGTTTGAAGTAGTATAGTCAATCA CTACTTCGACTC	Guide RNA of AtSK23
AtSK32_gRNA1_Fw	TTTTGAAGACATATTGTGCTGTATCGGAAAAACCCGGTTTTA GAGCTAGAAATAGC	Guide RNA of AtSK32
AtSK32_gRNA2_Rv	TTTTGAAGACTTAAACCGCGGTTGGCATTTCGCTCGCAATCA CTACTTCGACTC	Guide RNA of AtSK32
AtSK42_gRNA1_Fw	TTTTGAAGACATATTGACACTTCTAGTTCAGTTGATGTTTTAG AGCTAGAAATAGC	Guide RNA of AtSK42
AtSK42_gRNA2_Rv	TTTTGAAGACTTAAACTTATCTTCATCTCAAGCATACAATCA CTACTTCGACTC	Guide RNA of AtSK42
Sequencing		
At5g12150_Seq_RV1	CAAATTTACTTCACCTCCC	PHGAP1 gDNA sequencing
At5g12150_Seq_RV2	TTCCAACAACCGAAGACT	PHGAP1 gDNA sequencing
At5g12150_Seq_RV3	TGGGACTGGAGAAGAAGG	PHGAP1 gDNA sequencing
At5g12150_Seq_RV4	TGGCTTGAGCATTATTGG	PHGAP1 gDNA sequencing
At5g12150_Seq_RV5	GAATCTTCAACACCAAAACC	PHGAP1 gDNA sequencing
At5g12150_Seq_RV6	TGATCCTTTAGCAGGTGT	PHGAP1 gDNA sequencing
At5g12150_Seq_RV7	GCTTGCAAGTTGCTCTTGT	PHGAP1 gDNA sequencing
At5g12150_Seq_RV8	CTTGTCTGTGTTTCCTC	PHGAP1 gDNA sequencing
At5g19390_Seq_RV1	TGGACCACTCTTGAAAAC	PHGAP2 gDNA sequencing
At5g19390_Seq_RV2	TCCACTCGTACAAATCTTCA	PHGAP2 gDNA sequencing
At5g19390_Seq_RV3	TTCCACCTCTTCCACATC	PHGAP2 gDNA sequencing
At5g19390_Seq_RV4	CAATGAACATAATGCGAGC	PHGAP2 gDNA sequencing
At5g19390_Seq_RV5	TCAGGCCCACTATTTCCA	PHGAP2 gDNA sequencing
At5g19390_Seq_RV6	TTTTGTGGATCATCGCC	PHGAP2 gDNA sequencing
At5g19390_Seq_RV7	CGTTGTGCGAGTTCATTT	PHGAP2 gDNA sequencing
At5g19390_Seq_RV8	GAGAAGGAACCAAAATGAG	PHGAP2 gDNA sequencing
At5g19390_Seq_RV9	CCCCTTTACTATTTCTCC	PHGAP2 gDNA sequencing
GWM13-F	GTAAAACGACGGCCAGTCTTA	Entry clone sequencing
GWM13-R	CCAGGAAACAGCTATGACCAT	Entry clone sequencing
mCherry_Seq_R	TACATGAAGTGAAGGGACAG	Expression clone sequencing
mCherry_Seq_F	GTAAAACGACGGCCAGTCTTA	Expression clone sequencing
eGFP1_rv	GCTGAAGTTGTGCGCGTTTA	Expression clone sequencing
eGFP2_fw	CGACCACTACCAGCAGAACA	Expression clone sequencing

AtSK11_CRISPRseqFw	TTGTTGGTCACGGATCTTT	CRISPR plant sequencing
AtSK11_CRISPRseqRv	CGGTGAATGTAAGATAAGGC	CRISPR plant sequencing
AtSK12_CRISPRseqFw	ttctgggatcacctttattg	CRISPR plant sequencing
AtSK12_CRISPRseqRv	TGAAACACAACACCAAAGGA	CRISPR plant sequencing
AtSK13_CRISPRseqFw	AAGTGTTTAGAAACGGGGGA	CRISPR plant sequencing
AtSK13_CRISPRseqRv	TGTATGAGATGTTTGGCTCG	CRISPR plant sequencing
AtSK21_CRISPRseqFw	CTGCTGTAGTTGATGGACAT	CRISPR plant sequencing
AtSK21_CRISPRseqRv	TGACTTGATGAGTAAGAGGA	CRISPR plant sequencing
AtSK22_CRISPRseqFw	cattctcatacagtcatacc	CRISPR plant sequencing
AtSK22_CRISPRseqRv	CCGGGAACAGTATGGATGTA	CRISPR plant sequencing
AtSK23_CRISPRseqFw	TTCCAGgtatcattgtgctt	CRISPR plant sequencing
AtSK23_CRISPRseqRv	TCAACctacagaacatacag	CRISPR plant sequencing
AtSK32_CRISPRseqFw	CCATTTCTTCGGATCCTgta	CRISPR plant sequencing
AtSK32_CRISPRseqRv	gttagttgaatgacCTGCTT	CRISPR plant sequencing
AtSK42_CRISPRseqFw	gtgagtttgcattgttctct	CRISPR plant sequencing
AtSK42_CRISPRseqRv	aacCTGTCTTGATTGACCAT	CRISPR plant sequencing
Cas9-F	TAACTCTGTGGGATGGGCTGTGATC	CRISPR construct sequencing
Cas9-R	GTGAGTCCGAGAGAGAGAGCGATC	CRISPR construct sequencing
RB_Seq_F	ttaccgccaatatatactg	CRISPR construct sequencing
pML1_Seq1	gagtttaattaacaatgataa	ML1 promoter sequencing
pML1_Seq2	ctttccaataagacgagt	ML2 promoter sequencing
pML1_Seq3	gtcctccaactacattagt	ML3 promoter sequencing
pML1_Seq4	tagaatctaactagcttatt	ML4 promoter sequencing
pML1_Seq5	tttaaatgatgaactgtaaa	ML5 promoter sequencing
pML1_Seq6	tgtttgtaaatgaatgagttt	ML6 promoter sequencing
qPCR		
PHGAP1-qPCR-Fw	CGACAGCATCACTTAAGTTTAC	qPCR of PHGAP1
PHGAP1-qPCR-Rv	TAGGCTAGTTTCATCCGTTCTC	qPCR of PHGAP1
PHGAP2-qPCR-Fw	CATTCCCACGAAAATCGAATGA	qPCR of PHGAP2
PHGAP2-qPCR-Rv	CTAAATCACATTACCAGCCAG	qPCR of PHGAP2
ATSK11-f	GGAGCAAGAGACTCTACTGGTGT	qPCR of AtSK11
ATSK11-r	CCACTGTCGCTTCCATTCT	qPCR of AtSK11
ATSK12-f	GGCAATGTGACTGAGACTGG	qPCR of AtSK12
ATSK12-r	TCCGCCATGTAAGTATTGT	qPCR of AtSK12
ATSK13-f	CGGCGGTGACTTTTCAGA	qPCR of AtSK13
ATSK13-r	GCCATAGAAGAAGCTGGTAATGTT	qPCR of AtSK13
Actin2_F	GATGAGGCAGGTCCAGGAATC	qPCR reference gene
Actin2_R	AACCCAGCTTTTAAAGCCTTT	qPCR reference gene

Table S1. Oligonucleotides used in this study. Related to STAR Methods.