SUMO/deSUMOylation of the BRI1 brassinosteroid receptor modulates plant growth responses to temperature

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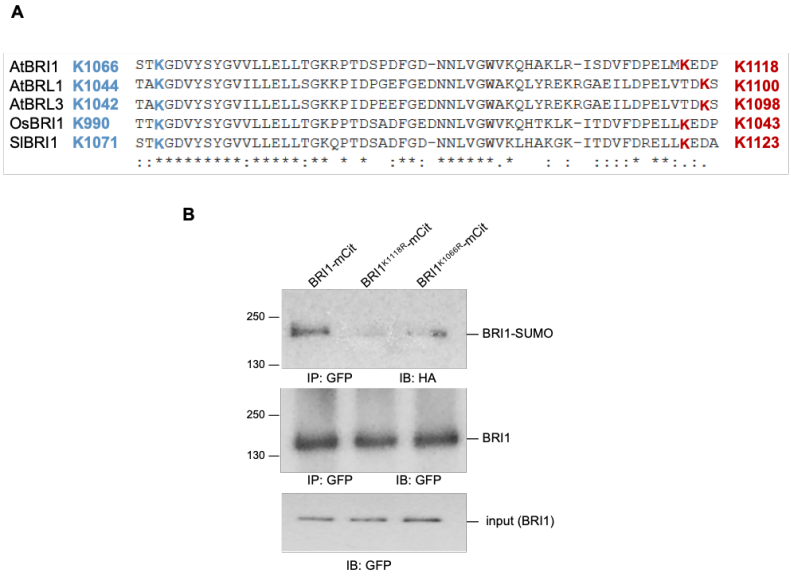

Figure S1. BRI1 is SUMOylated in vivo on two intracellular lysine residues.

A. Sequence alignment of the cytosolic domains of BRI1 (amino acids 1064-1121) and its homologs BRL1 and BRL3. Predicted SUMO targets in BRI1 and homologs are shown. B. In vivo SUMOylation analyses of BRI1, $BRI1_{K1066R}$ and $BRI1_{K1118R}$. BRI1-mCit, $BRI1_{K1066R}$-mCit and $BRI1_{K1118R}$-mCit were transiently expressed in *N. benthamiana* leaves prior to immunoprecipitation using anti-GFP antibodies on solubilized protein extracts. All constructs were co-expressed with SUMO1-HA. Detection of immunoprecipitated proteins used the anti-SUMO1 and anti-GFP antibodies.

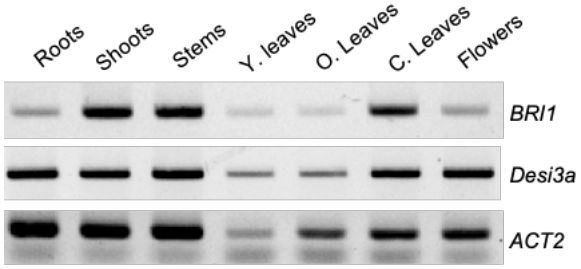

Figure S2. *Desi3a* and *BRI1* expression profiles overlap in plants.

Semi-quantitative RT-PCR analyses of *Desi3a* and *BRI1* mRNA accumulation in different tissues of wild-type plants.

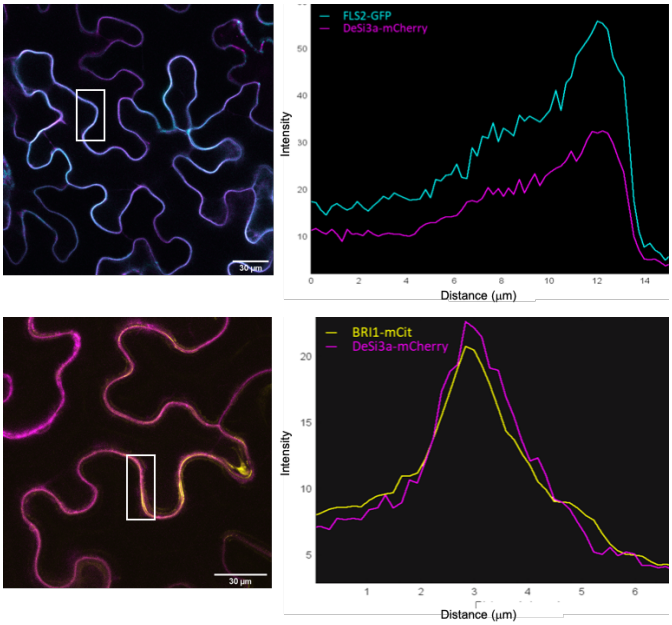

Figure S3. Colocalization of Desi3a with the plasma membrane-localized receptors FLS2 and BRI1.

Fluorescence intensity profiles of Desi3a-mCh with FLS2-GFP (top) and BRI1-mCit (bottom), respectively. The regions of interest used to monitor fluorescence profiles are shown.

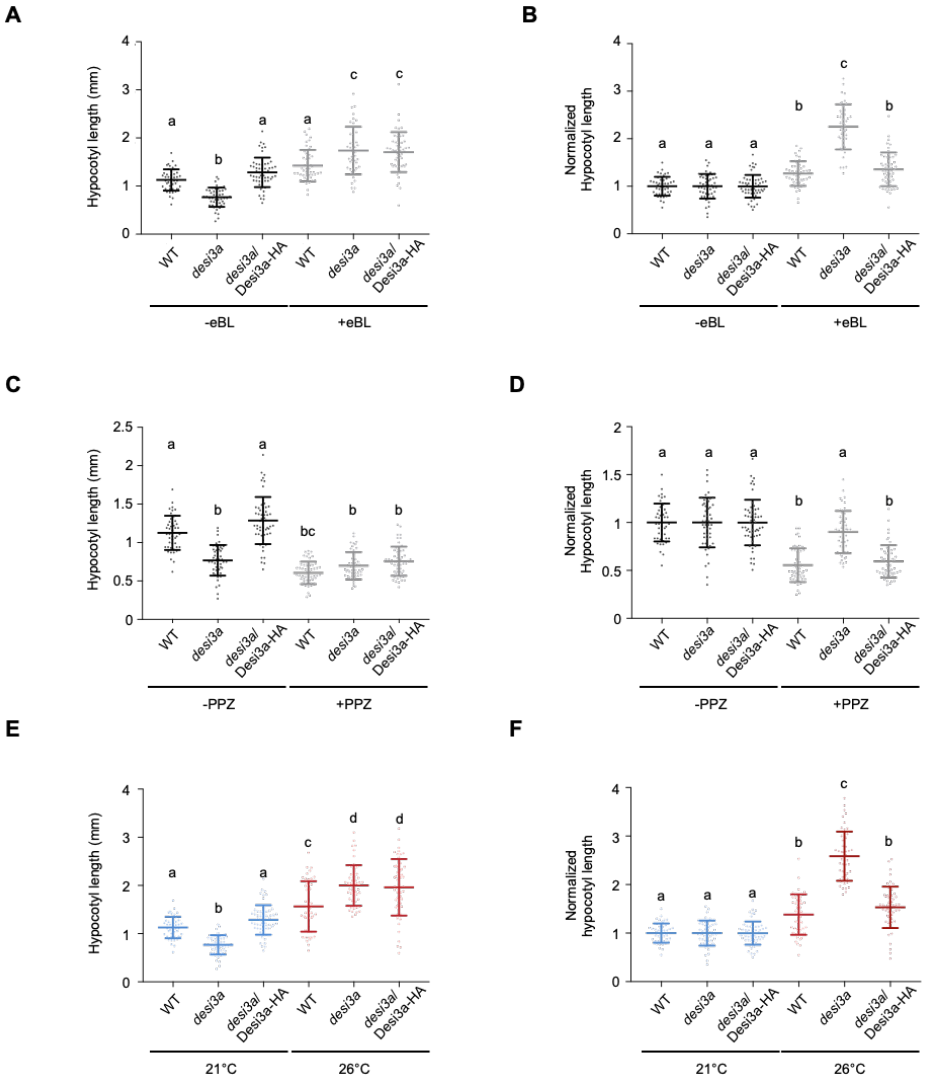

Figure S4. Phenotype of the *desi3a*/Desi3a-HA complemented line upon BR treatment and temperature elevation.

A. Hypocotyl length of 6-day-old wild-type (WT), *desi3a*, and *desi3a*/Desi3a-HA complemented plants grown on plates containing mock or 500 nM epibrassinolide (eBL). Experiments were carried out in triplicates. Error bars represent SD (n=40). Statistical differences were calculated by two-way ANOVA with Tukey's multiple comparison test. B. Normalized hypocotyl length from wild-type (WT) *desi3a*, and *desi3a*/Desi3a-HA plants grown as in A. Experiments were carried out in triplicates. Error bars represent SD (n=40). Statistical differences were calculated by two-way ANOVA with Tukey's multiple comparison test. C. Hypocotyl length of 6-day-old wild-type (WT), *desi3a*, and *desi3a*/Desi3a-HA

complemented plants grown on plates containing mock or 2μM propiconazole (PPZ). Experiments were carried out in triplicates. Error bars represent SD (n=40). Statistical differences were calculated by two-way ANOVA with Tukey's multiple comparison test. D. Normalized hypocotyl length from wild-type (WT) *desi3a*, and *desi3a*/Desi3a-HA plants grown as in C. Experiments were carried out in triplicates. Error bars represent SD (n=40). Statistical differences were calculated by two-way ANOVA with Tukey's multiple comparison test. E. Hypocotyl length of 6-day-old wild-type (WT), *desi3a*, and *desi3a*/Desi3a-HA complemented plants grown at 21°C or 26°C. Experiments were carried out in triplicates. Error bars represent SD (n=40). Statistical differences were calculated by two-way ANOVA with Tukey's multiple comparison test. F. Normalized hypocotyl length from wild-type (WT) *desi3a*, and *desi3a*/Desi3a-HA plants grown as in E. Experiments were carried out in triplicates. Error bars represent SD (n=40). Statistical differences were calculated by two-way ANOVA with Tukey's multiple comparison test.

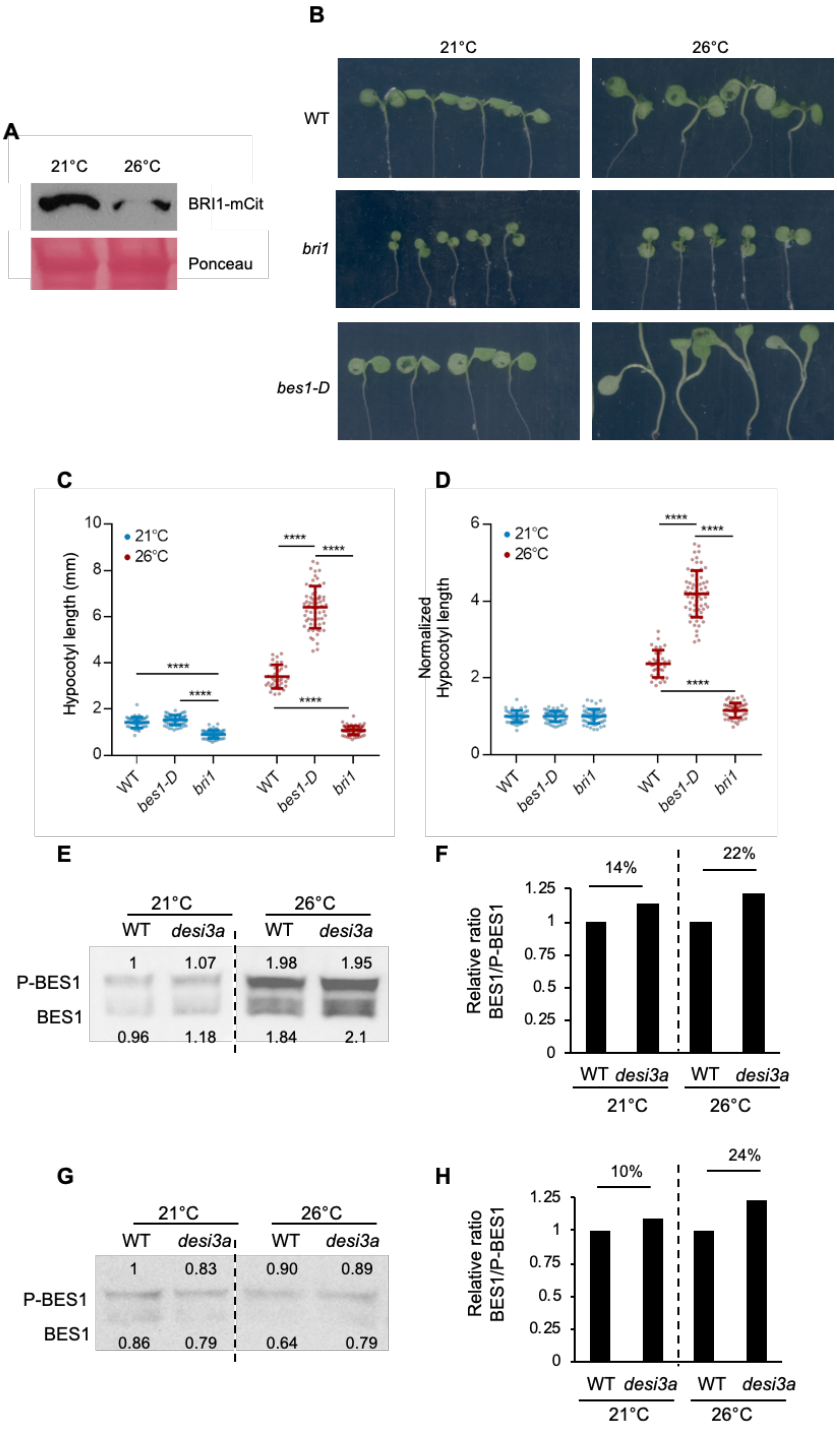

Figure S5. Impact of temperature elevation on BR signaling.

A. Influence of warm temperature on BRI1 protein levels. Western blot analyses monitoring BRI1 protein accumulation in BRI1-mCit-expressing plants grown at 21°C or 26°C using anti-GFP antibodies. Ponceau red is used as loading control. B. Phenotype of 6-day-old wild-type (WT), *bri1* and *bes1-D* mutant plants grown at 21°C or 26 °C in the light. C. Hypocotyl length

of 6-day-old wild-type (WT), *bri1* and *bes1-D* mutant plants grown as in B. Experiments were carried out in triplicates. Error bars represent SD (n=40). The asterisks indicate a statistically significant difference with wild-type plants (two-way ANOVA with Sidak's multiple comparison test). D. Ratio of hypocotyl length from wild-type (WT), *bri1* and *bes1-D* mutant plants grown at 21°C and 26 °C for 6 days. Experiments were carried out in triplicates. Error bars represent SD (n=40). The asterisks indicate a statistically significant difference with wild-type (two-way ANOVA with Sidak's multiple comparison test). E. First replicate of BES1 phosphorylation state in wild-type (WT) or *desi3a* plants grown at 21°C or 26 °C. Detection of BES1 was performed with anti-BES1 antibodies. Signal intensities of BES1 and P-BES1 are indicated. F. Normalized BES1 to phosphorylated BES1 (P-BES1) ratios from the western blot shown in E, relative to wild-type. G. Second replicate of BES1 phosphorylation state in wild-type (WT) or *desi3a* plants grown at 21°C or 26 °C. Detection of BES1 was performed with anti-BES1 antibodies. Signal intensities of BES1 and P-BES1 are indicated. H. Normalized BES1 to phosphorylated BES1 (P-BES1) ratios from the western blot shown in G, relative to wild-type.

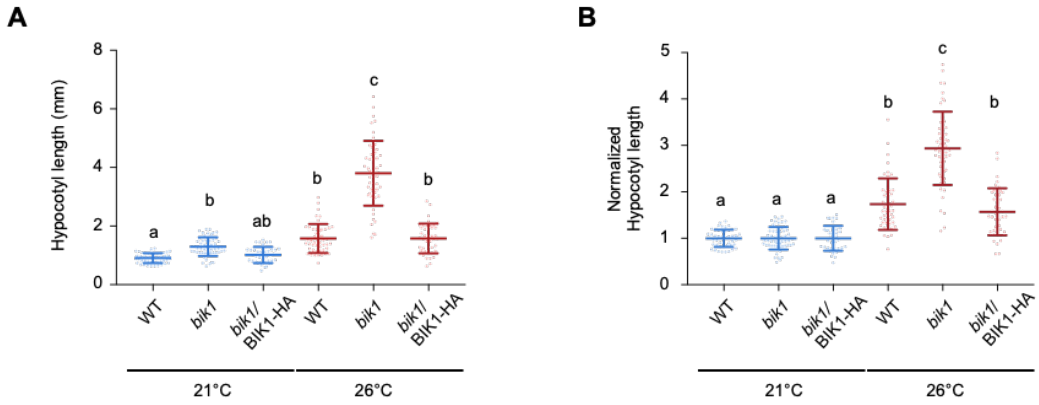

Figure S6. Phenotype of the *bik1*/BIK1-HA complemented line at 21°C and 26°C.

A. Hypocotyl length of 6-day-old wild-type (WT), *bik1* and *bik1*/BIK1-HA complemented line grown at 21°C or 26 °C in the light. Experiments were carried out in triplicates. Error bars represent SD (n=40). Statistical differences were calculated by two-way ANOVA with Tukey's multiple comparison test. B. Normalized hypocotyl length of 6-day-old wild-type (WT), *bik1* and *bik1*/BIK1-HA complemented line grown as in A. Experiments were carried out in triplicates. Error bars represent SD (n=40). Statistical differences were calculated by two-way ANOVA with Tukey's multiple comparison test.

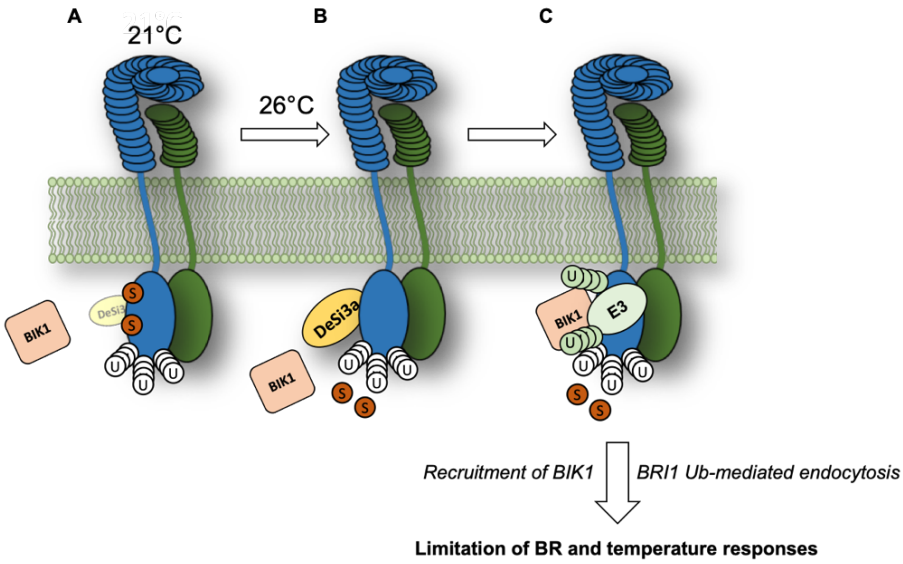

Figure S7. Model for Desi3a function in the control of BR signaling at 21°C and 26°C.

A. At standard growth temperature (21°C), Desi3a SUMO protease levels are low and BRI1 is SUMOylated at residues K1066 and K1118. B. At warmer growth temperature (26°C), Desi3a accumulates leading to the deSUMOylation of BRI1. C. DeSUMOylated BRI1 shows increased interaction with the negative regulator of the brassinosteroid signaling pathway BIK1 and promotes BRI1 internalization from the plasma membrane. These two parallel mechanisms downregulate BRI1, thus preventing plants from over-growing in response to temperature elevation. We postulate that the increased internalization and degradation of BRI1 observed at 26°C results from the ubiquitination of BRI1 at residues K1066 and K1118.

Table S1. Primers used in this study

Name	Sequence	Purpose
BRI1-K1066R-F	GGTGTTCAACAAGAGGAGACGTTTATAGTTACGG	SDM
BRI1-K1066R-R	CCGTAACTATAAACGTCTCCTCTTGTTGAACACC	SDM
BRI1 K1118R F	CGAGCTTATGAGGGAAGATCCAG	SDM
BRI1 K1118R R	CTGGATCTTCCCTCATAAGCTCG	SDM
DESI3a_qPCR_F	CATACTTCCCGAGTCCCTCA	RT-PCR
DESI3a_qPCR_R	AGTTGCCAAGGAGGTAAGCA	RT-PCR
BRI1 RT-PCR F	TCCGCGGTGTGATCCTTCAAAT	RT-PCR
BRI1 RT-PCR R	GCCGTGTGGACCAGTTTAGTTT	RT-PCR
ACT2 F	GCCCAGAAGTCTTGTTCCAG	RT-PCR
ACT2 R	TCATACTCGGCCTTGGAGAT	RT-PCR