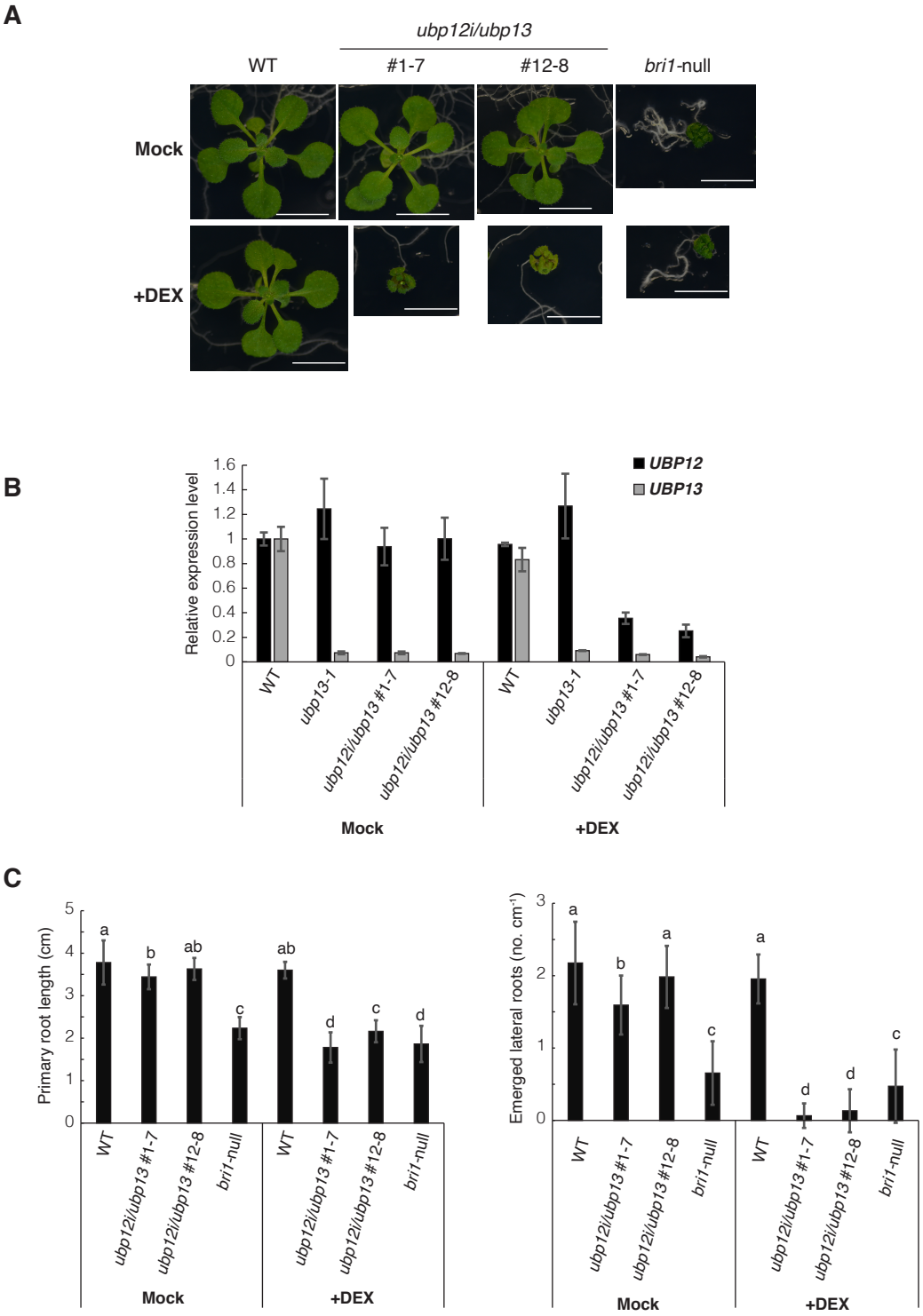
Appendix Content



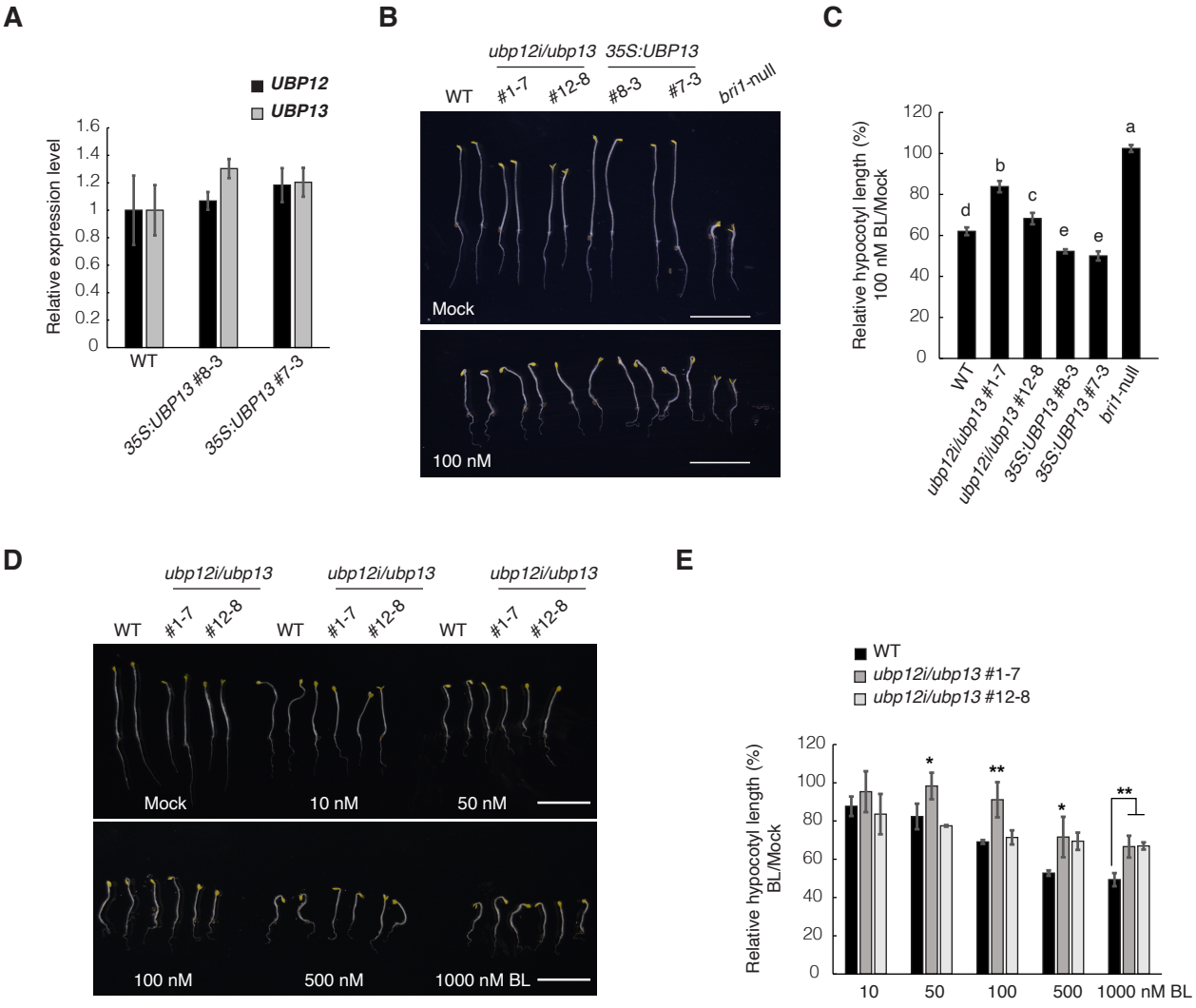
Appendix Figure S1. Dwarf phenotype of the *ubp12i/ubp13* double mutant

A Growth phenotypes of the wild-type (WT), *bri1*-null mutant, and *ubp12i/ubp13* double mutant grown on 1/2MS medium supplemented with 10 μM DEX (+DEX) or without DEX (Mock) for 18 days. Scale bars: 10 mm.

B Transcript levels of the *UBP12* and *UBP13* genes in the double mutants. Total RNA was isolated from 6-day-old seedlings grown on 1/2MS medium supplemented with 10 μM DEX or not (Mock). The expression levels of *UBP12* and *UBP13* were assessed by qRT-PCR relative to that in WT grown on mock medium. The expression of *ACTIN7* was used as an internal control (n = 3 biological replicates).

C Root growth defects in the *ubp12i/ubp13* double mutant. Seedlings were grown on 1/2MS medium supplemented with 10 μM DEX or not (mock) for 11 days and used for measurement of primary root lengths (left) and emerged lateral root densities (right) (n > 20 seedlings for each line).

Data information: data are presented as means ± SD (B and C). $P<0.05$ (one-way ANOVA and post-hoc Tukey's test) (C).



Appendix Figure S2. Loss of UBP12 and UBP13 decreased BR sensitivity, whereas overexpressing *UBP13* led to BR hypersensitivity in *Arabidopsis*

A Transcript levels of the *UBP12* and *UBP13* genes in the *35S:UBP13* lines. Total RNA was isolated from 6-day-old dark-grown seedlings. The expression levels of *UBP12* and *UBP13* were assessed by qRT-PCR relative to that in WT. The expression of *18S rRNA* was used as an internal control (n = 3 biological replicates).

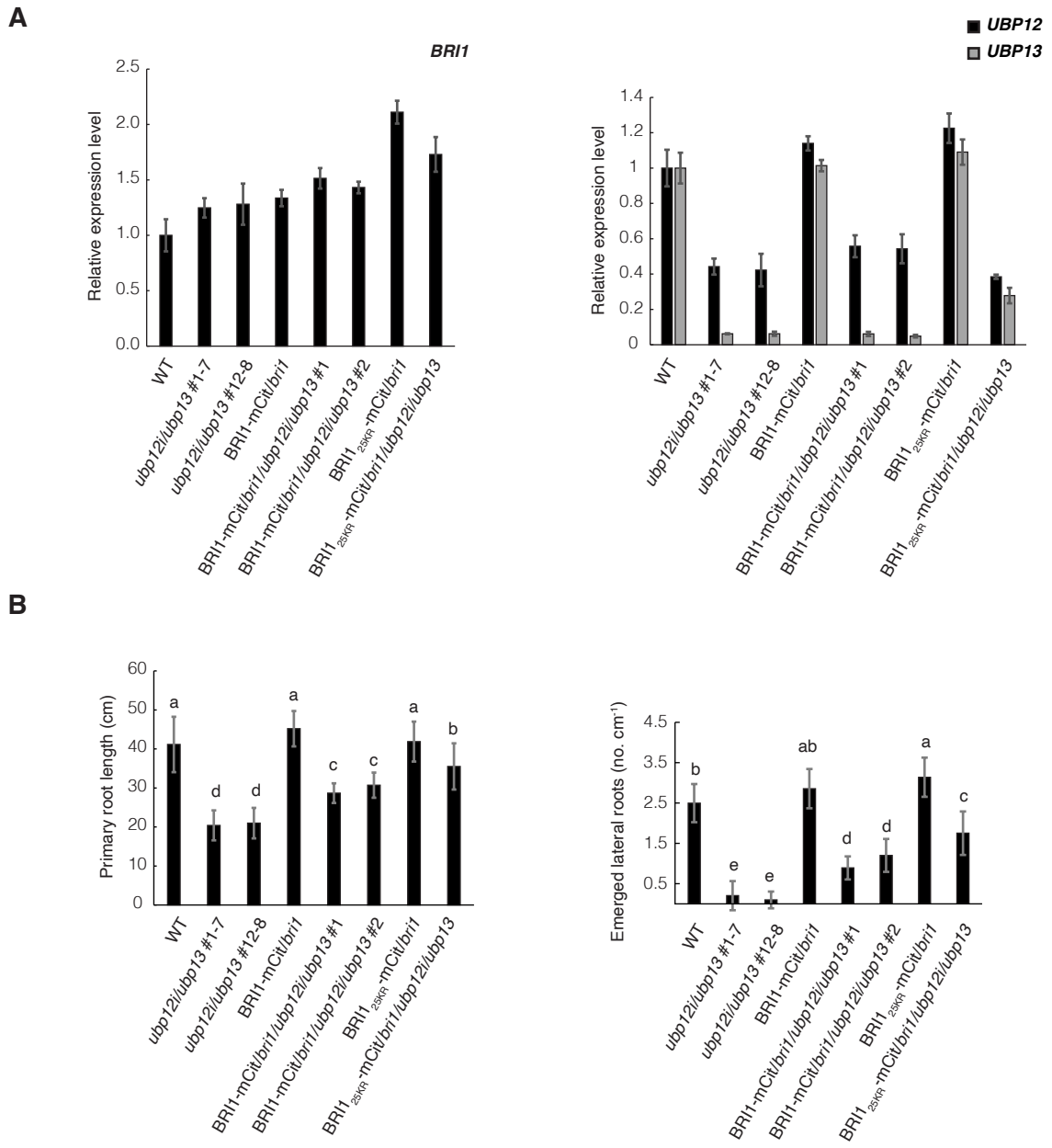
B Phenotype of WT, *ubp12i/ubp13* double mutant, two independent lines of *35S:UBP13*, and *bri1*-null mutant grown on DEX medium in the presence of 100 nM BL or not (Mock) in the dark for 6 days. Scale bars: 10 mm.

C Hypocotyl length relative to the mock control of seedlings (B). Experiments were done in triplicate (n > 15 seedlings for each line).

D Phenotype of the WT and *ubp12i/ubp13* double mutant grown on DEX medium in the presence of increasing concentrations of BL in the dark for 6 days. Scale bars: 10 mm.

E Hypocotyl length relative to the mock control of seedlings (D). Experiments were done in triplicate (n >15 seedlings for each line).

Data information: data are presented as means ± SD (A, C, and E). $P<0.05$ (one-way ANOVA and post-hoc Tukey's test) (C), $*P<0.05$, $**P<0.01$ compared with WT under each condition (one-way ANOVA and post-hoc Tukey's test) (E).

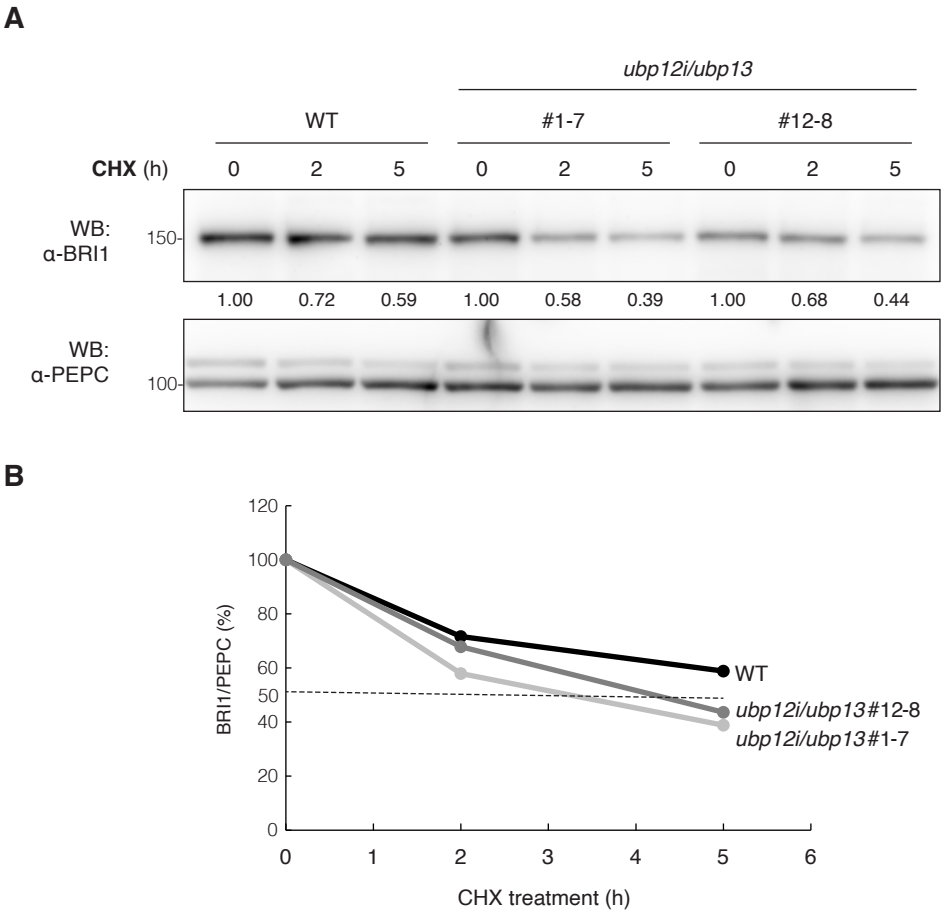


Appendix Figure S3. Characterization of BRI1-mCit/*bri1/ubp12i/ubp13* and BRI1$_{25KR}$-mCit-/*bri1/ubp12i/ubp13*

A Transcript levels of the *BRI1*, *UBP12*, and *UBP13* genes. Total RNA was isolated from 7-day-old seedlings grown on DEX medium. The expression levels of *BRI1*, *UBP12* and *UBP13* were assessed by qRT-PCR relative to that in WT. The expression of *18S rRNA* was used as an internal control (n = 3 biological replicates).

B Root growth defects in transgenic lines in the *ubp12i/ubp13* background. Seedlings were grown on DEX medium for 11 days and used for measurement of primary root lengths (left) and emerged lateral root densities (right) (n > 20 seedlings for each line).

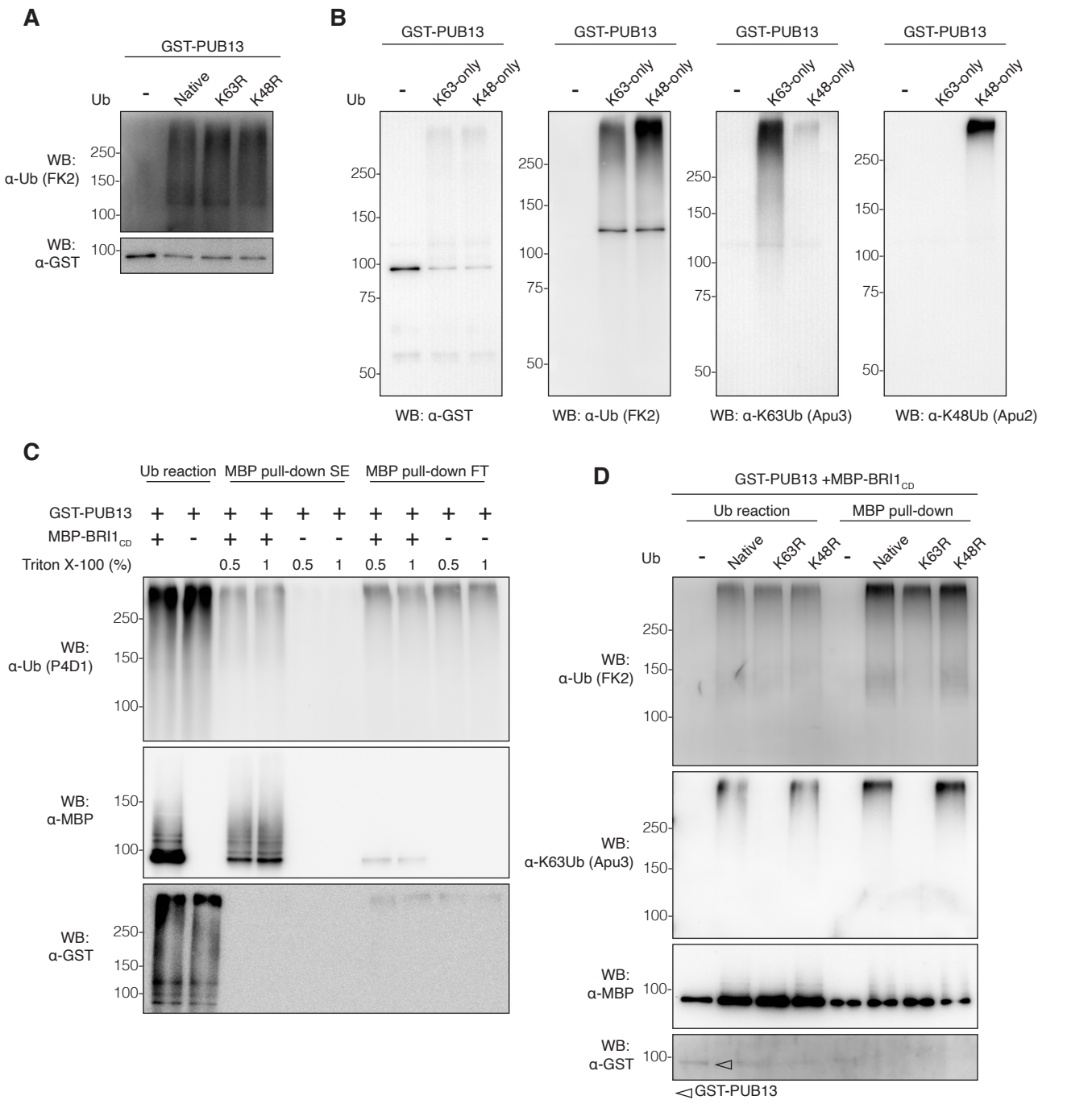
Data information: data are presented as means ± SD. P<0.05 (one-way ANOVA and post-hoc Tukey's test) (B).



Appendix Figure S4. BRI1 protein stability decreases in *ubp12i/ubp13* double mutant

A Eighteen-day-old plants grown on DEX medium transferred to 1/2MS liquid medium with 200 µM CHX in the presence of 10 µM DEX for the indicated time. BRI1 proteins were analyzed by western blot with an α-BRI1 antibody. PEPC was used as a loading control. The values shown above each lane indicate the abundance of the BRI1 proteins relative to that of samples before CHX treatment in the respective lines.

B Degradation rates of BRI1 proteins (A) plotted over time. The relative protein abundance of BRI1 proteins before CHX treatment was set to 100% in the respective lines (n = 1 biological replicate).

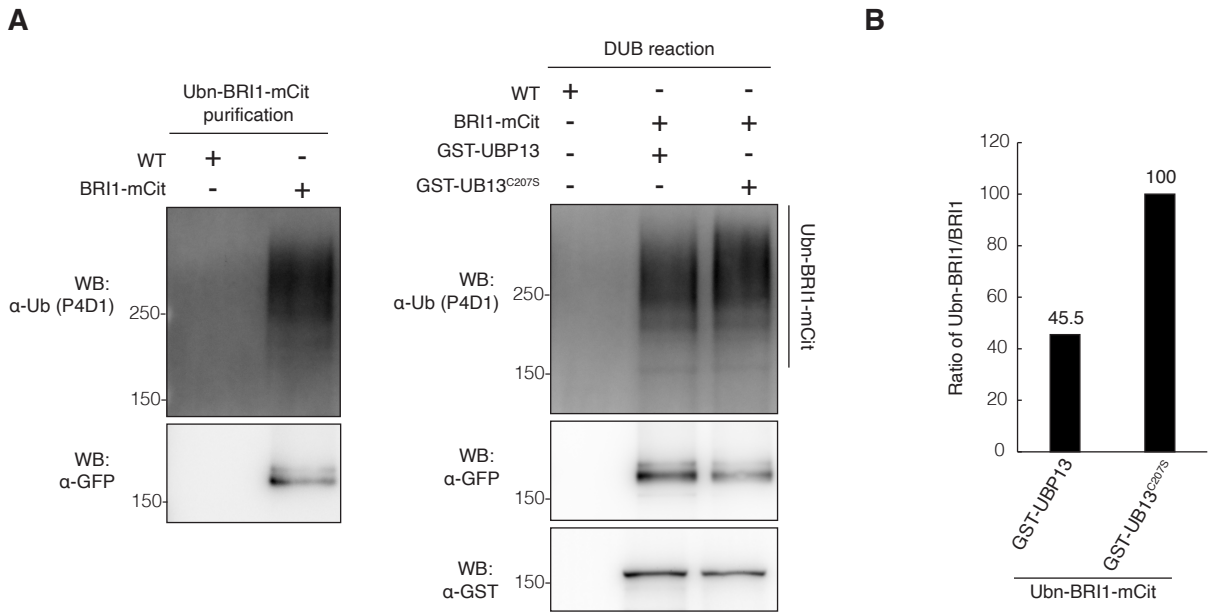


Appendix Figure S5. PUB13 catalyzes K63-linked polyubiquitin chains on BRI1

A,B K63- and K48-autoubiquitination activities of PUB13 *in vitro*. GST-PUB13 was incubated without (-) or with native ubiquitin (Native), K63R, or K48R ubiquitin (A) and K63- or K48-only ubiquitin (B). PUB13 ubiquitination was analyzed by western blot with α-Ub (FK2), α-K63Ub (Apu3), α-K48Ub (Apu2), and α-GST antibodies.

C Purification of polyubiquitinated MBP-BRI1$_{CD}$ by MBP pull-down assay. *In vitro* ubiquitination assays produced polyubiquitinated MBP-BRI1$_{CD}$ and polyubiquitinated GST-PUB13 mixture or polyubiquitinated GST-PUB13 alone (Ub reaction) were incubated with amylose resin to purify MBP-BRI1$_{CD}$ (MBP pull-down). Resins were washed in wash buffer containing 0.5% or 1% (v/v) Triton X-100 after incubation for dissociation of nonspecific bindings (flow through [FT]). Polyubiquitinated MBP-BRI1$_{CD}$ eluted from the amylose resin by SDS sample buffer (SE). The ubiquitinated proteins were detected by western blot with an α-Ub (P4D1) antibody. The presence of recombinant proteins was confirmed by α-GST and α-MBP antibodies.

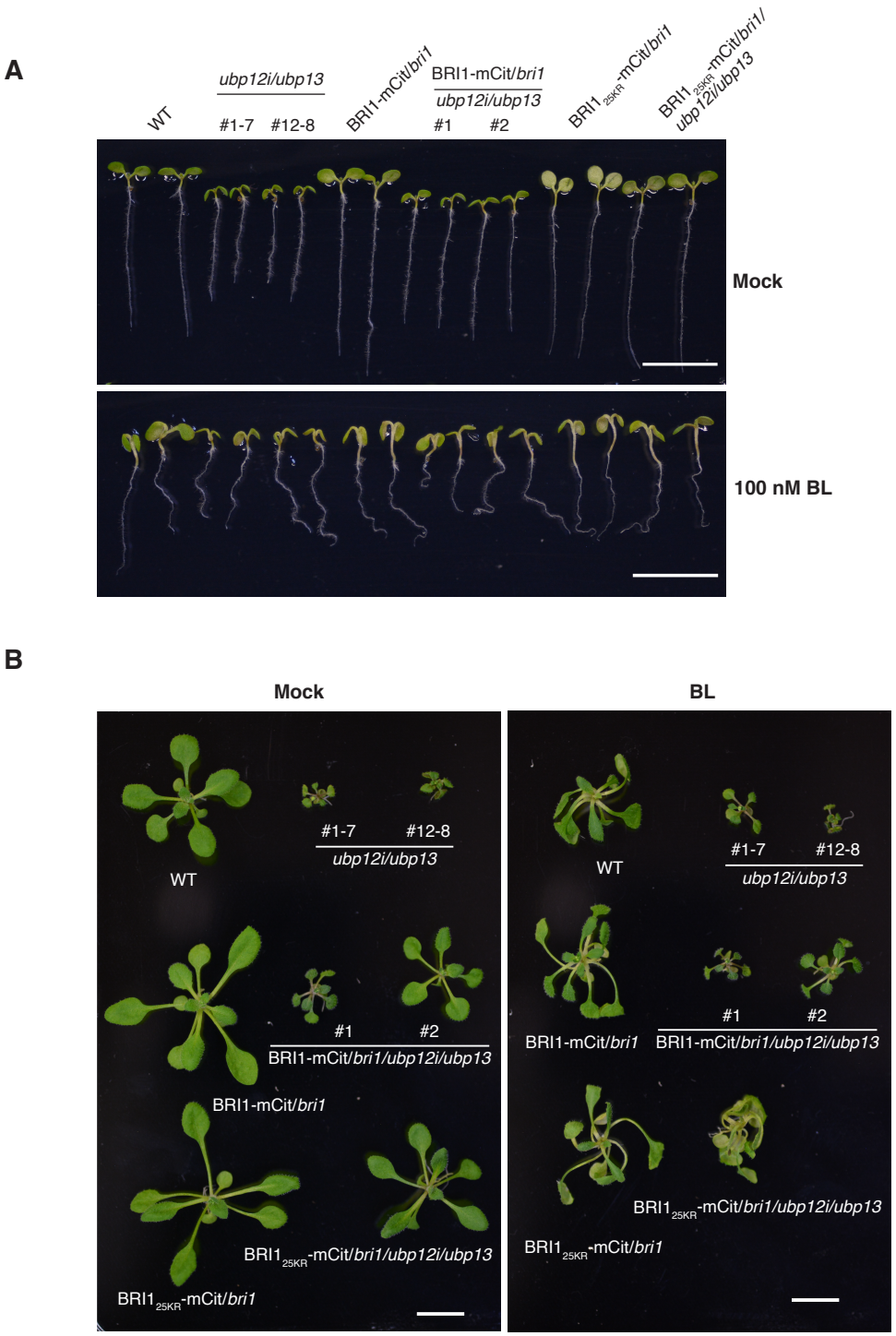
D K63-ubiquitination of BRI1 by PUB13 *in vitro*. MBP-BRI1$_{CD}$ was incubated with GST-PUB13 in the presence of native ubiquitin or ubiquitin variants or in the absence of ubiquitin (-). Polyubiquitinated MBP-BRI1$_{CD}$ was purified by MBP pull-down assay and analyzed by western blot with α-Ub (FK2) and α-K63Ub (Apu3) antibodies.



Appendix Figure S6. Deubiquitination of BRI1 expressed in *Arabidopsis* plants by recombinant UBP13

A Cell-free deubiquitination assay of polyubiquitinated BRI1-mCit proteins by GST-UBP13. Polyubiquitinated BRI1-mCit proteins were extracted from 18-day-old BRI1-mCit/bri1 plants (left) and incubated with GST-UBP13 or GST-UBP13^{C207S} for 10 h (right). BRI1 ubiquitination was analyzed by western blot with α-Ub (P4D1) and α-GFP antibodies. The presence of GST-UBP13 or GST-UBP13^{C207S} recombinant proteins was confirmed by the α-GST antibody.

B Quantification of BRI1 ubiquitination profiles from the band intensities (A). Data are presented as ratios of ubiquitinated BRI1 (P4D1)-to-basal BRI1 (GFP), shown as numbers above each bar. The ratio of sample incubated with GST-UBP13^{C207S} was set to 100 (n = 1 biological replicate).



Appendix Figure S7. BL-responsive phenotypes of BRI1-mCit/*bri1/ubp12i/ubp13* and BRI1$_{25KR}$-mCit-/*bri1/ubp12i/ubp13*

A Phenotype of transgenic lines expressing BRI1 or BRI1$_{25KR}$ tagged with mCitrine in either the UBP12/UBP13 or *ubp12i/ubp13* background grown on DEX medium in the presence of 100 nM BL or not (mock) in the light for 6 days. Scale bars: 10 mm.

B Growth phenotype of the indicated lines grown on DEX medium for 10 days, then transferred to fresh DEX medium containing 1 µM BL or mock solution (0.1% [v/v] EtOH) for additionally 8 days. Scale bar: 10 mm.

Appendix Table S1. Primers used in this study

	Primer name	Sequence (5'→3')
Cloning	UBP13 ent 5-1	CACCATGACTATGATGACTCCGC
	UBP13 ent 3-1	CTAATTGTATATTTTCACCGGCTTCTCG
	UBP12RNAi Si_R	CACCTATACCACATACCTTACTTCAGAAAG
	UBP12RNAi Si R	AAAGAAGCATAAACATCCTTGCAG
	BRI1 ent 5-1	CACCATGAAGACTTTTTCAAGCTTC
	BRI1 ent 3-1	TAATTTTCCTTCAGGAACTTC
	UBP13 C207S 5	GGTGCTACCAGTTACATGAATT
	UBP13 C207S 3	AATTCATGTAACTGGTAGCACC
	CIPK8 ent 5-1	CACCATGGTGGTAAGGAAGGT
	CIPK8 ent 3-1	CAAGGCCAAGAGTAAAAGACGT
Genotyping	ubp13-1 LP	GTCTGGCTGTAGTGAATCAAGTC
	ubp13-1 RP	TAATGCCCTCCATGCACTCC
	bri1 LP	GAATCTATCCGCTTCGTTGC
	bri1 RP	AATTCGACCGAGCCCGAAAA
qRT-PCR	UBP13 exp FP	GTGCGTGGGAGCAGTATCTT
	UBP13 exp RP	GCGTGTCGGTTCTGATTTG
	UBP12 exp FP	TCTGGACATCCCTCTTCCAG
	UBP12 exp RP	CGACCGTGCTTTGTTTAGGTA
	BRI1 exp FP	CAACTGCAGTCCGCATATCA
	BRI1 exp RP	AAACCCGAGCTTCCAAATTC