Author Correction: Downstream nuclear events in brassinosteroid signaling

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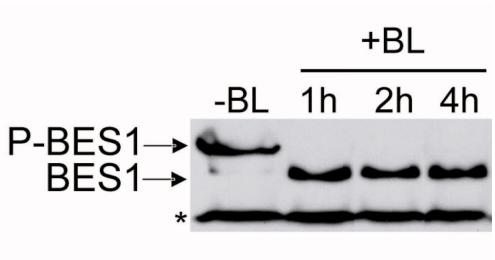
In the Letter "Downstream nuclear events in brassinosteroid signalling", the following mistakes were made during figure mounting.

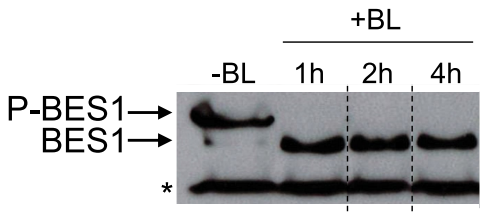
The published Fig. 3a depicts a western blot experiment monitoring the phosphorylation status and levels of BES1 at different time points (1, 2, 4 hours) after treatment with brassinolide (BL) or mock. Figure 3a contains undisclosed cropping to organize samples differently from the original experiment and to show a single untreated control, as no change in BES1 phosphorylation or levels was observed with time in mock-treated plants. In addition, an inversion of lanes between the 2 h and 4 h +BL signals occurred during figure preparation. We have retrieved the original blot (shown in the Supplementary Information) and have produced a corrected panel for Fig. 3a in which cropped regions are shown with dotted lines (Corrected Fig. 3a). The correction does not alter the original conclusions that BL treatment leads to dephosphorylation of the BES1 transcription factor, with little or no change in total BES1 levels in our conditions.

The published Fig. 3b shows a western blot experiment for BES1 in different genetic backgrounds following mock or BL treatments, as well as exposure of wild-type (WT) plants to the BL biosynthetic inhibitor brassinazole (BRZ) to deplete endogenous BRs. The original blot was modified to remove background shadows above P-BES1 signals without mention in the corresponding legend. We have recovered the original blot (shown in the Supplementary Information), and have produced a new panel supporting the prominent role of BL in controlling BES1 phosphorylation status, with no obvious change in the accumulation of BES1 protein in our conditions (Corrected Fig. 3b below). Additionally, the signal corresponding to WT plants grown on BRZ results from the duplication of the BL-deficient *det2*-1 background lane. The fact that BES1 accumulates under its P-BES1 form in absence of BL and that BL addition promotes the dephosphorylation of P-BES1 into BES1 had already been observed in several independent studies[1–3], and by us in other unpublished experiments showing the effect of BL and BRZ now shown below (Unpublished Fig. 1). The western blot shown on the corrected Fig. 3b therefore lacks the WT+BRZ lane and, consistently, the phenotype of a BRZ-grown plant was removed. We apologize for such alteration of the blot. The conclusions of this experiment remain unchanged.

Figure 3a, as published in the original paper



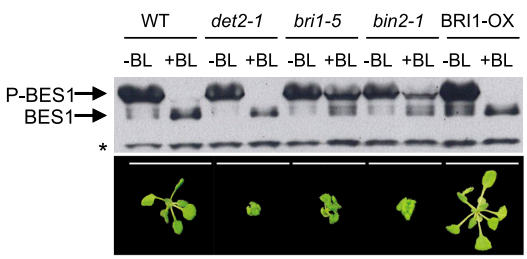
Corrected Figure 3a



Corrected Fig. 3a | Time course analyses of BL treatment on BES1 protein. Plants were treated with mock (-BL) or BL (+BL) for the time indicated and subjected to western blot analysis using anti-BES1 antibody. The blot was cropped to show the -BL 1 h control only. The dotted lines indicate cropping. PBES1, phosphorylated BES1; *, non-specific band.

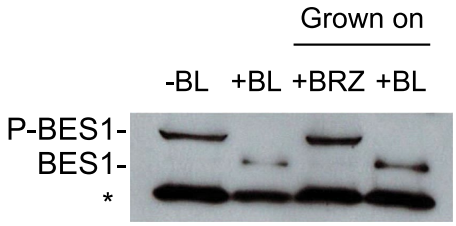
Figure 3b, as published in the original paper

Corrected Figure 3b



Corrected Fig. 3b | BES1 is constitutively nuclear and its levels are unaffected by BRs. BES1 phosphorylation status in response to BL treatment in wild-type (WT) plants or genotypes affected in BR biosynthesis (*det2-1*) or signalling (*bri1-5, bin2-1* and a BRI1-OX plant which overexpresses BRI1(ref. 27 [ref. 6 below]). Plants were treated with mock or BL as described in the legend to Fig. 1d and subjected to western blot analysis using anti-BES1 antibody. P-BES1, phosphorylated BES1; *, non-specific band. The phenotype of the corresponding plants is shown. Only the wild-type Col is shown, as both Col and Ws ecotypes responded in the same way to BL treatment (data not shown).

Unpublished blot showing the BL and BRZ effect



Unpublished Fig. 1 | BRZ promotes BES1 phosphorylation. BES1 phosphorylation status in response to BL or BRZ treatments in wild-type (WT). Plants were treated with mock (-BL) or BL (+BL) as described in the legend to Fig. 1d, or grown on 1 µM BRZ (Grown on +BRZ) or 1 µM BL (Grown on +BL), and subjected to western blot analysis using anti-BES1 antibody. PBES1, phosphorylated BES1; *, non-specific band.

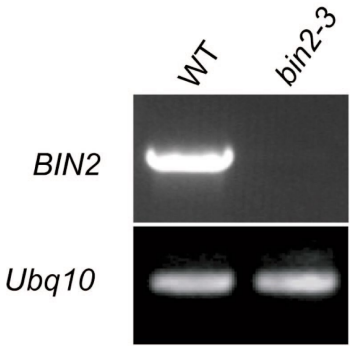
There are several additional duplications:

1. Duplications of *Ubq10* RT-PCR DNA loading control gels between Supplementary Fig. S1b and Fig. S5 (lanes 1, 2), and between Supplementary Fig. S3c and Fig. S5 (lanes 1, 2; lanes 4, 5, respectively).
2. Possible duplications of lanes 1, 2 between the two subpanels of Supplementary Fig. S3c.
3. Duplications in Supplementary Fig. S5: lanes 3 and 4, BIN2-GFP; and lanes 4 and 5, actin.

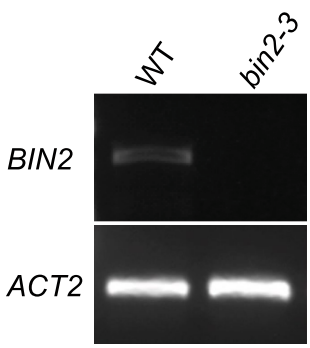
Careful examination of other RT-PCR DNA gels highlighted some similarities for *Ubq10* between the two panels of Supplementary Fig. S2b.

The seeds corresponding to most lines failed to germinate, except for *bin2-3*, for which we were able to repeat the experiment presented in the published Supplementary Fig. S1b (full DNA gels are found in the Supplementary Information of this correction file). We therefore built a new panel and confirmed our original conclusions that the *bin2-3* allele is a knock-out mutant for the *BIN2* gene, and that BIN2 acts as a negative regulator of brassinosteroid signalling (Corrected Supplementary Fig. S1b). In contrast to WT plants, no amplification of *BIN2* was indeed observed using cDNAs from *bin2-3* while the *ACT2* loading

Supplementary Fig. S1b, as published in the original paper

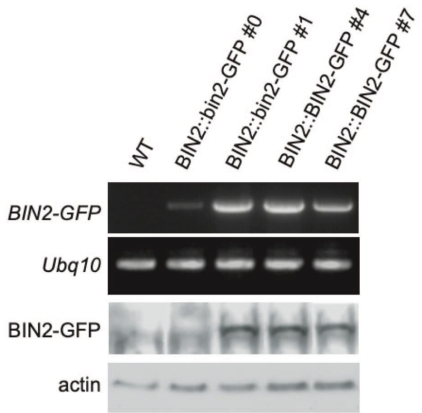


Newly generated data corresponding to the claims of the original Supplementary Fig. S1b

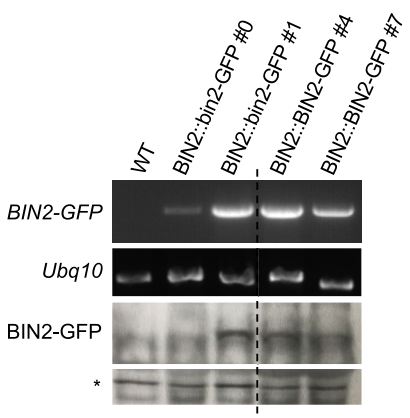


Corrected Fig. S1b | Loss-of-function of BIN2 displays constitutive BR responses. Detection of *BIN2* mRNA. RT-PCR was performed on total mRNA extracted from whole seedlings using *BIN2* specific primers. *ACTIN2* (*ACT2*) amplification served as a control.

Supplementary Fig. S5, as published in the original article



Data corresponding to the original data in Supplementary Fig. S5; and the new data for loading control



Corrected Fig. S5 | Expression analysis of transgenic lines expressing BIN2-GFP and bin2-GFP. Expression of BIN2-GFP and bin2-GFP transgenes. RT-PCRs using BIN2- and GFP-specific primers were performed on several transgenic lines carrying either BIN2-GFP or bin2-GFP constructs. Amplification of *Ubq10* was used as loading control. BIN2-GFP and bin2-GFP lines were also subjected to western blot analysis with a GFP antibody. Transgenic lines harboring similar levels of RNA and protein (BIN2::BIN2-GFP #4 and the dwarf line BIN2::bin2-GFP #1) were selected for further analyses. The dotted line indicates cropping. *, non-specific band used for loading control.

control was amplified in both backgrounds. The *ACT2* primers used are 5′-GCCCAGAAGTCTTGTTCCAG-3′ and 5′-TCATACTCGGCCTTGGAGAT-3′.

The published Supplementary Fig. S5 depicts the accumulation of BIN2-GFP or bin2-GFP (carrying the E263K gain-of-function mutation) proteins and corresponding mRNA, to confirm that the transgenic lines used expressed transgenes to comparable levels. The protein blot probed with GFP antibodies, aiming at detecting both BIN2-GFP and bin2-GFP, was cropped to remove a lane corresponding to a BIN2-GFP transgenic line that failed to accumulate the corresponding protein and that in addition had a severe phenotype unrelated to brassinosteroids. A similar cropping was performed on the corresponding *BIN2-GFP* RT-PCR DNA gel. We apologize for not having clearly described such croppings in the corresponding legend. We found the original BIN2-GFP protein blot and *BIN2-GFP* DNA gel (shown in the Supplementary Information) and have highlighted cropped regions with dotted lines. The published *Ubq10* DNA corresponded to a different replicate experiment and has therefore been replaced by the appropriate one in the corrected Supplementary Fig. S5. Additionally, when building the published figure, lane 3 (BIN2::bin2-GFP#1) from the BIN2-GFP protein blots was accidentally used for lanes 4 and 5 (BIN2::BIN2-GFP#4 and #7) as well as lane 4 from the actin blot duplicated as lane 5. The actin loading control blot could not be recovered. We have therefore built a corrected figure panel below, using a non-specific band as loading control (Corrected Supplementary Fig. S5).

The authors apologize deeply for the inconvenience caused. The abovementioned corrections do not change the main conclusions of the published article. In addition, the findings reported in our original article regarding the role of BIN2 and related GSK3 kinases in brassinosteroid signalling, as well as the role of BIN2 phosphorylation in regulating DNA-binding of BES1-related transcription factors, have been fully validated by independent groups[4,5].

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Supplementary information is available in the online version of this amendment.