Extended Data Inventory

Local brassinosteroid biosynthesis enables optimal root growth

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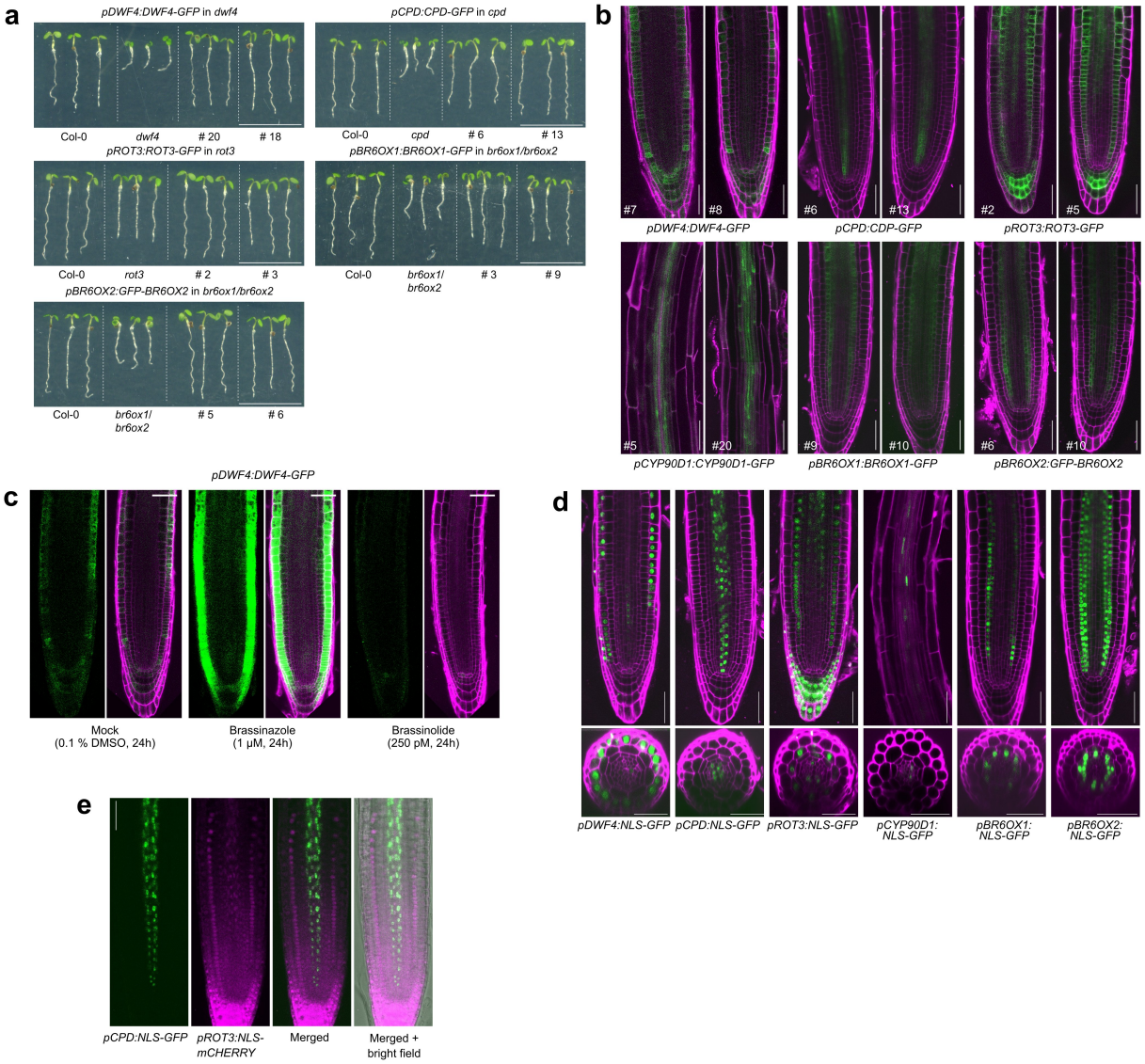
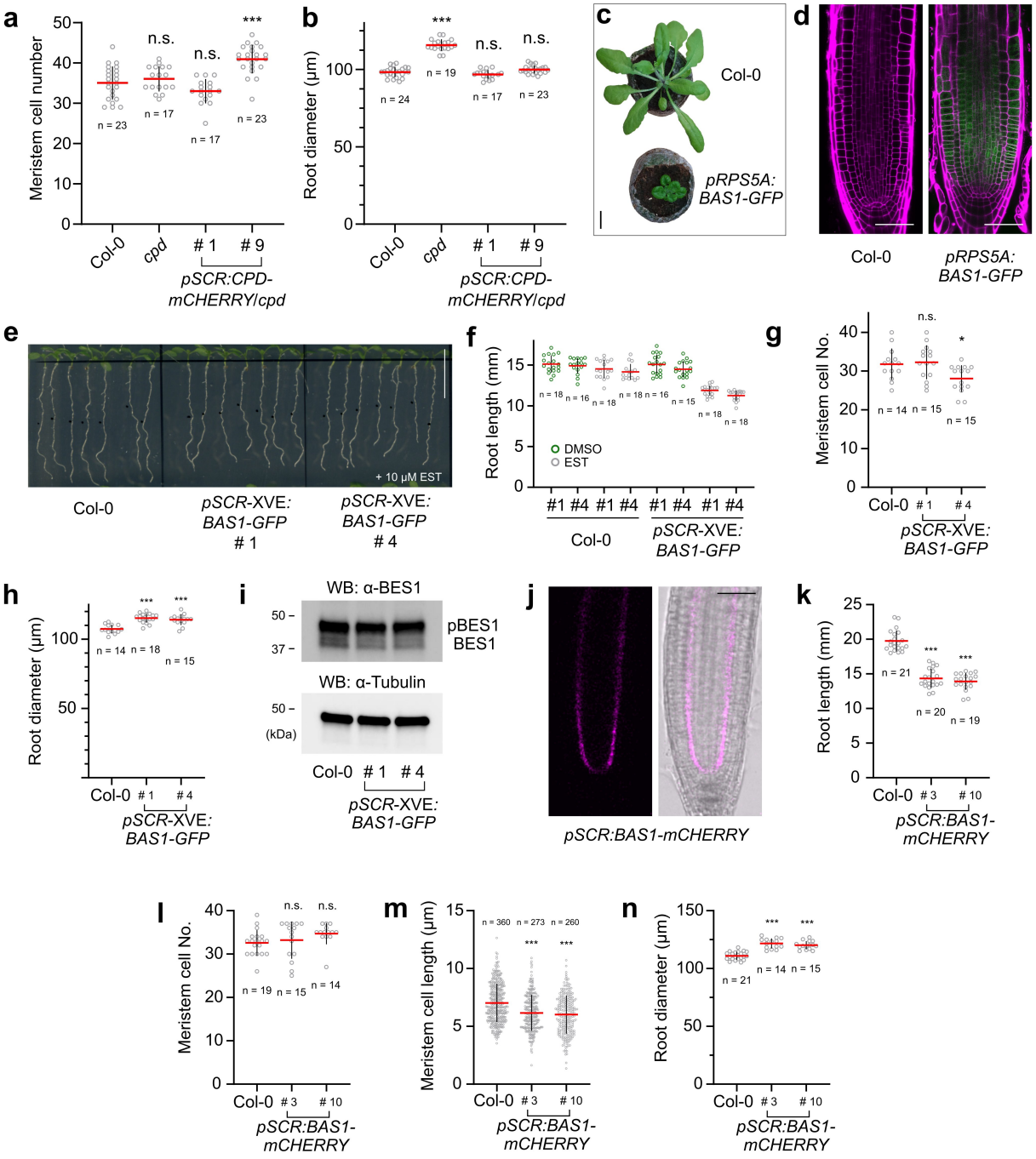


Extended Data Fig. 1. The GFP-tagged BR biosynthetic enzymes are functional and show similar expression patterns in multiple independent transgenic lines. **a**, Rescue of the BR biosynthetic mutants by expression of the corresponding GFP-tagged enzymes under the control of their endogenous promoters. Two independent transgenic lines for each gene are compared with the wild type (Col-0) and the corresponding mutant. Six-day-old seedlings are shown. Scale bars, 1 cm. **b**, Consistent expression patterns of GFP-tagged BR biosynthetic enzymes under the control of their endogenous promoters in multiple independent transgenic lines. Root meristems of the 6-day-old seedlings from two independent transgenic lines for each GFP-tagged enzyme are shown. For seedlings expressing *pCYP90D1:CYP90D1-GFP* only the root elongation zone is shown, since the signal in the root apical meristem is extremely weak. Roots were stained with propidium iodide. Scale bars, 50 µm. **c**, Six-day-old *pDWF4:DWF4-*

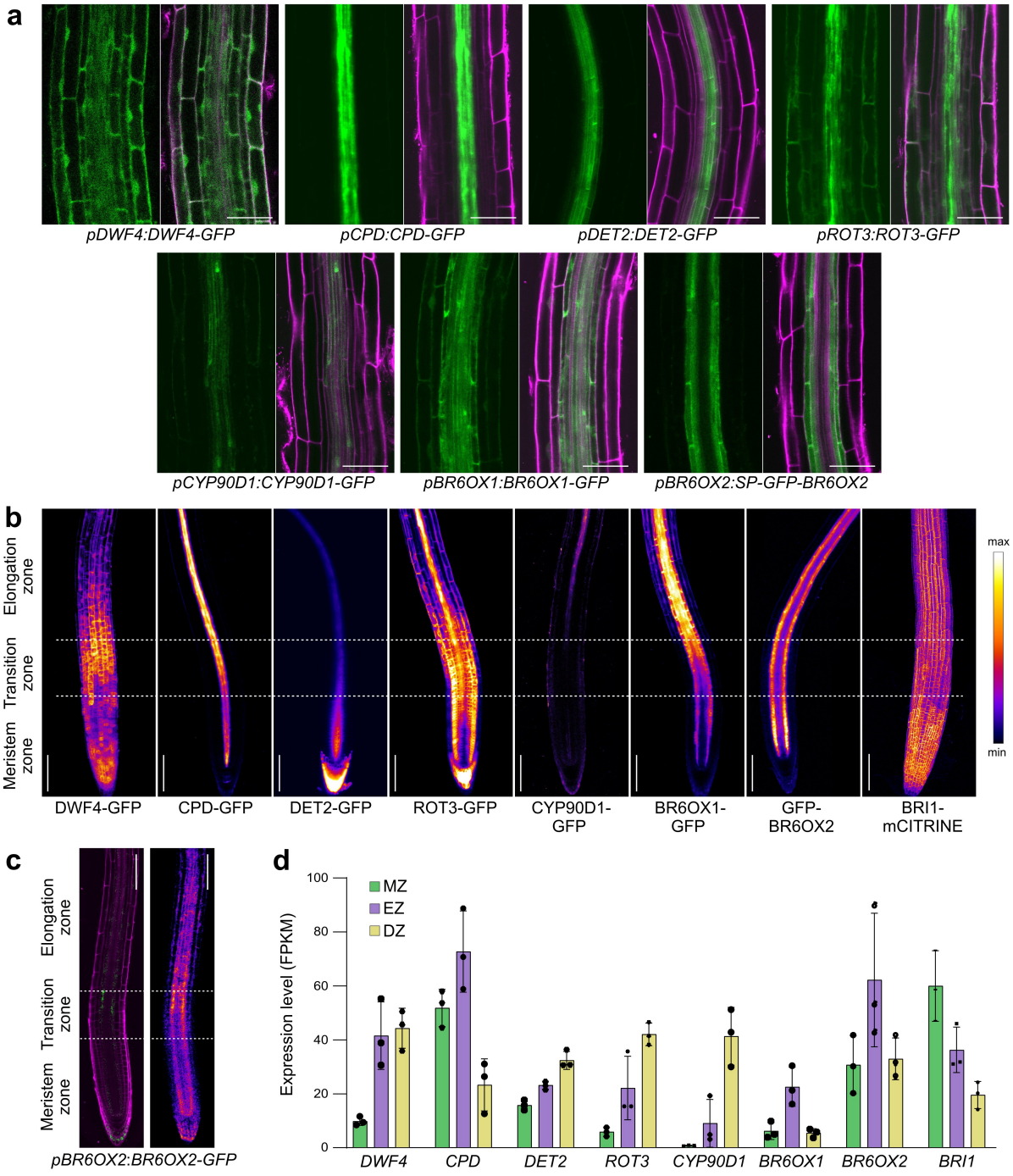
GFPdwf4 roots treated with mock (DMSO), brassinazole and brassinolide for 24 h. Scale bars, 50 µm. **d**, Expression patterns of the *PROMOTER-NLS-GFP* reporters for all BR biosynthetic genes. For each reporter line, 6-day-old root meristems and cross section are shown. Scale bars, 50 µm. **e**, Co-expression of *NLS-GFP* and *NLS-mCHERRY* reporters under the control of *CPD* and *ROT3* promoters, respectively. Scale bar, 50 µm. Roots were stained with propidium iodide.



Extended Data Fig. 2. Ectopic expression of CPD and BAS1 in the *Arabidopsis* root. a, Quantification of the root meristem cell number of *cpd* seedlings expressing the *pSCR:CPD-mCHERRY* construct. All individual data points are plotted. Red horizontal bars represent the means and error bars represent s.d. *n* = number of roots analysed. The significant differences between transgenic lines and the Col-0 control were determined by one-way analysis of variance (ANOVA) and Dunnett's multiple comparisons tests. *** $P < 0.001$, ** $P < 0.01$ and * $P < 0.05$. The *P* value versus the Col-0 control for *cpd* is = 0.7077, line # 1 = 0.1849 and line

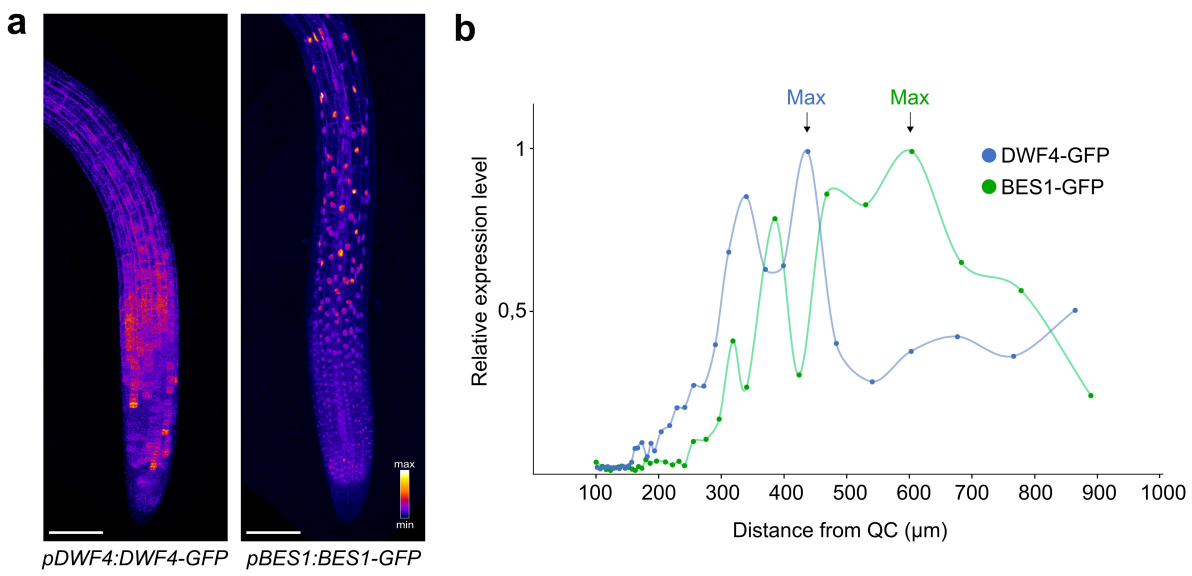
9 < 0.0001. The experiment was repeated independently three times with similar results. **b**, Reverted root meristem diameter of the *cpd* mutant to that of the wild type upon ectopic expression of *CPD* in the endodermis. Six-day-old seedlings of the two independent transgenic lines were measured. All individual data points are plotted. Red horizontal bars represent the means and error bars represent s.d. *n* = number of roots analysed. The significant differences between transgenic lines and the Col-0 control were determined by one-way analysis of variance (ANOVA) and Dunnett's multiple comparisons tests. *** $P < 0.001$, ** $P < 0.01$ and * $P < 0.05$. The *P* value versus the Col-0 control for *cpd* is < 0.0001, line # 1 = 0.3417 and line # 9 = 0.1597. The experiment was repeated independently three times with similar results. **c**, Active BR depletion by the BAS1-GFP overexpression causing a typical BR deficient phenotype. Scale bar, 1 cm. **d**, Thickening of the meristem and shortening of the meristem cells caused by the BAS1-GFP overexpression, typical for BR-related mutants. More than three independent experiments were performed. Representative images are shown. Scale bars, 50 μm. **e**, Decreased root growth by the inducible, tissue-specific expression of BAS1-GFP in the endodermis. Two independent transgenic lines were compared with the wild type (Col-0). Four-day-old seedlings were transferred to fresh medium containing 10 μM β-estradiol (EST) and imaged after 3 days. Scale bar, 1 cm. f, Non-induced pSCR-XVE:BAS1-GFP seedlings do not show impaired root growth. n = number of roots analysed. g, h, Quantification of meristem cell number (g) and root diameter (h) of EST-induced pSCR-XVE:BAS1-GFP seedlings. All individual data points are plotted. Red horizontal bars represent the means and error bars represent s.d. n = number of roots analysed. The significant differences between transgenic lines and the Col-0 control were determined by one-way analysis of variance (ANOVA) and Dunnett's multiple comparisons tests. *** $P < 0.001$, ** $P < 0.01$ and * $P < 0.05$. The P values versus the Col-0 control for transgenic lines are as follows: for (g) line # 1 = 0.9250 and line # 4 = 0.0294; for (h) line # 1 < 0.0001and line # 4 < 0.0001. More than three independent experiments were performed. Representative images are shown. i, BES1 phosphorylation status tested by western blot with the α-BES1 antibody in roots of 7-day-old Col-0 and pSCR-XVE:BAS1-GFP seedlings. Tubulin was used as a loading control. Seedlings were germinated and grown on medium containing 10 μM β-estradiol. The experiment was performed twice with similar outcome. j, Confocal image of a 6-day-old root meristem expressing pSCR:BAS1-mCHERRY. More than three independent experiments were performed. Representative images are shown. Scale bar, 50 μm. k-n, Quantification of root length (k) meristem cell number (l), meristem cortical cell length – first 20 cells of individual roots (m) and root diameter (n) of Col-0 and pSCR:BAS1-mCHERRY 6-day-old seedlings. All individual data points are plotted.

Red horizontal bars represent the means and error bars represent s.d. n = number of roots analysed for (k), (l) and (n) and n = number of cells analysed for (m). For (m) number of individual roots used in experiment are 18, 14 and 13 for Col-0, line # 3 and line # 10, respectively. The significant differences between transgenic lines and the Col-0 control were determined by one-way analysis of variance (ANOVA) and Dunnett's multiple comparisons tests. *** $P < 0.001$, ** $P < 0.01$ and * $P < 0.05$. The P values versus the Col-0 control for transgenic lines are as follows: for (**k**) line # 3 < 0.0001 and line # 10 < 0.0001; for (**l**) line # 3 = 0.8157 and line # 10 = 0.1336; for (**m**) line # 3 < 0.0001 and line # 10 < 0.0001; for (**n**) line # 3 < 0.0001 and line # 10 < 0.0001. All experiments were repeated independently more than three times with similar results.

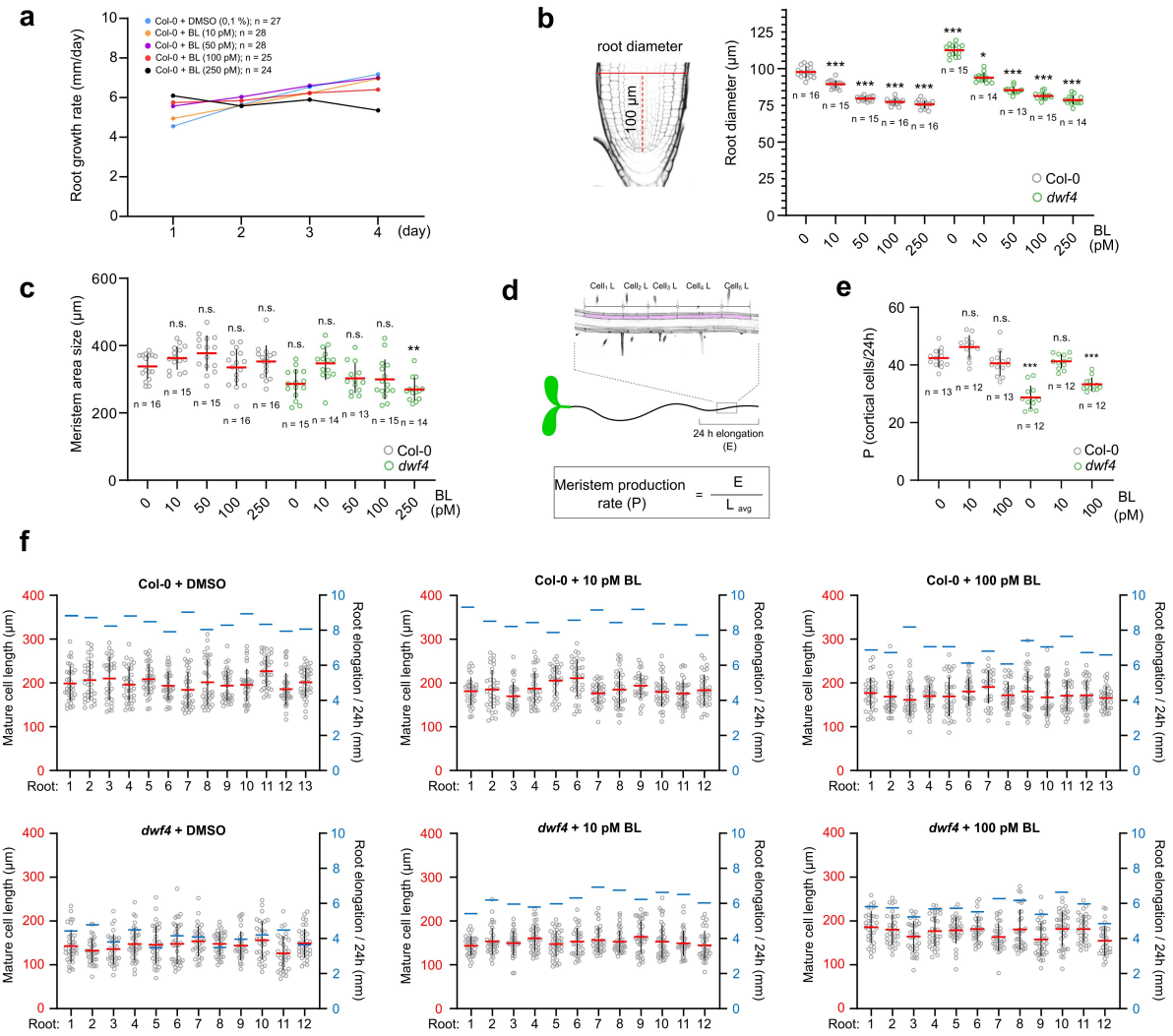


Extended Data Fig. 3. Expression patterns of BR biosynthetic enzymes in the root elongation zone. a, The expression domains of several genes expanding in the root elongation zone. DWF4-GFP expression is very broad and the GFP signal can be observed in all cell types of the elongation zone. The CPD-GFP expression is restricted to the stele of the elongation zone. The DET2-GFP expression is limited to the stele, similarly to the expression of CPD-GFP. The ROT3-GFP expression has maxima in the stele, but a signal also occurs in all other

cell types. The expression of the ROT3 homolog, CYP90D1, is limited to the stele. BR6OX1 and BR6OX2 both have expression maxima in endodermis and pericycle, but the expression pattern of BR6OX1 is expanded in all cell types. Roots were stained with propidium iodide. Scale bars, 50 μm. **b**, Increase in the expression levels of most of the BR biosynthetic enzymes in the transition and elongation zones as visualized by colour-based fluorescence intensity coding. All reporter lines showed an increase in expression higher in the root except for DET2-GFP and GFP-BR6OX2. The signal intensity of the BR receptor, BRI1-mCITRINE in pBRI1:BRI1-mCITRINE/bri1 line did not exhibit signal increase in the elongation zone. Maximum Z projections of the same roots from Fig. 2a are shown for BR biosynthetic enzyme lines. Scale bars, 100 μm. c, Roots expressing pBR6OX2:BR6OX2-GFP (left panel). Increased GFP signal can be observed in elongation zone of as visualized by colour-based fluorescence intensity coding (right panel). Scale bars, 100 μm. All transgenic lines in (a), (b) and (c) were imaged in more than three independent experiments. Representative images are shown. d, Expression levels of BR biosynthetic genes and BRI1 in the Arabidopsis root. All BR biosynthetic genes show an increased expression in the elongation zone, while BRI1 exhibits highest expression in meristem. Extracted from Huang and Schiefelbein (2015). MZ, meristematic zone; EZ, elongation zone; DZ, differentiation zone. Symbols depict individual values. Bars represent s.d..

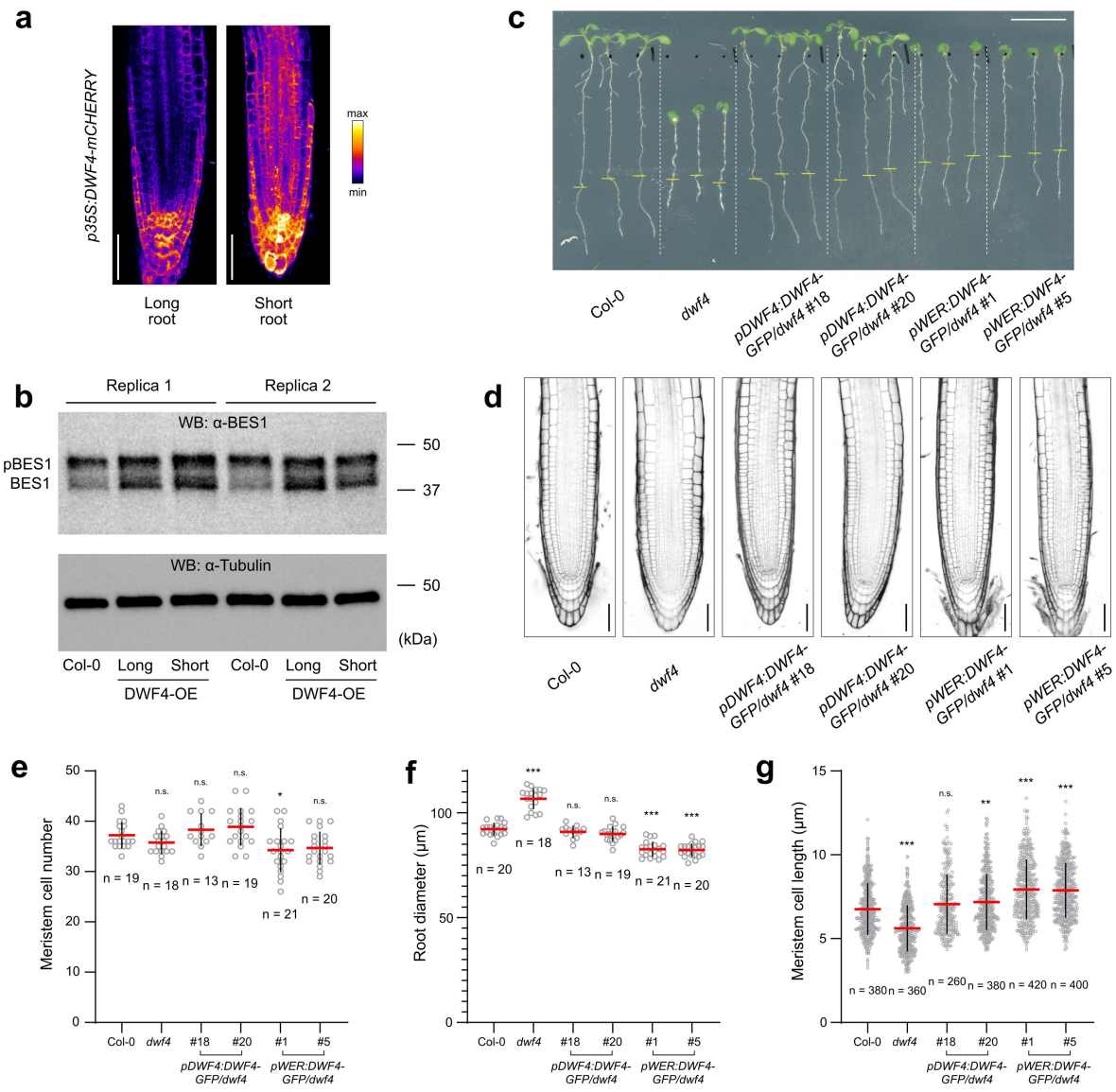


Extended Data Fig. 4. The *DWF4* expression maximum precedes the BR signalling peak monitored by the nuclear fluorescence intensity of BES1-GFP. a, Six-day-old *Arabidopsis* roots expressing DWF4-GFP/*dwf4* and BES1-GFP/Col-0 under the control of their native promoters. Scale bars, 100 µm. **b**, Fluorescence intensity of DWF4-GFP and BES1-GFP in single cells within single cell files, along the longitudinal root axis shown in **a**. Note that the DWF4-GFP expression peak precedes the BES1-GFP nuclear accumulation peak in elongation zone. The DWF4-GFP fluorescence intensity was measured for the whole cells along the trichoblast file whereas the BES1-GFP florescence intensity was measured in the nuclei along cortical file.



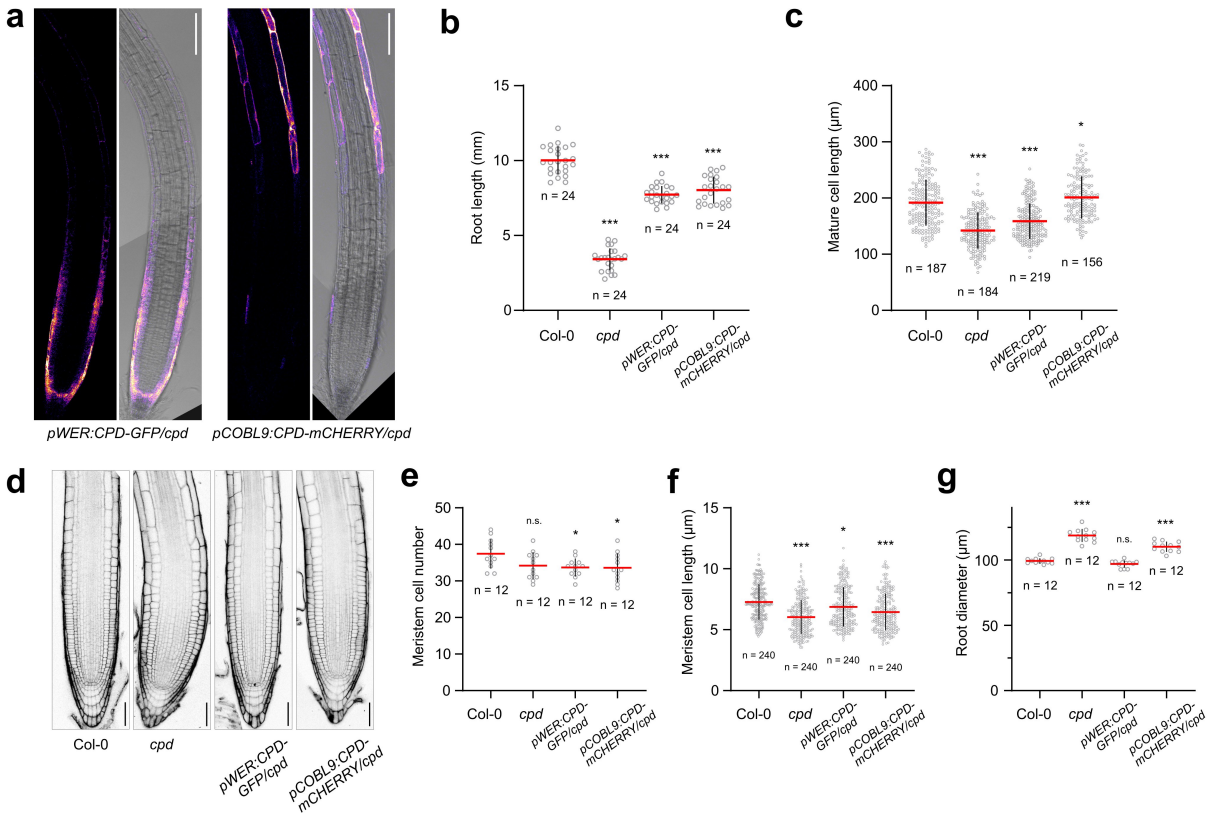
Extended Data Fig. 5. Effects of exogenous brassinolide (BL) on root growth. a, Root growth rates of Col-0 plants grown on increasing concentrations of BL. Six-day-old seedlings were transferred to fresh media containing BL or mock and root growth was followed for 4 days. Values represent the means of the root growth rates ± s.d.. *n* = number of roots analysed.. **b**, **c,** Quantification of the root meristem diameter (**b**) and the root meristem area size measured from the quiescent center to the first elongated cell (**c**) of Col-0 and *dwf4* treated with increasing concentrations of BL. Roots were treated with BL for 24 h. All individual data points are plotted. Red horizontal bars represent the means and error bars represent s.d. *n* = number of roots analysed. The significant differences between Col-0 and the *dwf4* seedlings treated with BL and Col-0 grown on mock (0) were determined by two-way analysis of variance (ANOVA) and Tukey's multiple comparisons tests. *** $P < 0.001$, ** $P < 0.01$ and * $P < 0.05$. The *P* values versus the Col-0 + DMSO control for different treatments are as follows: for (**b**) Col-0

+ 10 pM BL < 0.0001, Col-0 + 50 pM BL < 0.0001, Col-0 + 100 pM BL < 0.0001, Col-0 + 250 pM BL < 0.0001, *dwf4* + DMSO < 0.0001, *dwf4* + 10 pM BL = 0.0142, *dwf4* + 50 pM BL < 0.0001, *dwf4* + 100 pM BL < 0.0001, *dwf4* + 250 pM BL < 0.0001; for (**c**) Col-0 + 10 pM BL = 0.9019, Col-0 + 50 pM BL = 0.3871, Col-0 + 100 pM BL > 0.9999, Col-0 + 250 pM BL = 0.9972, *dwf4* + DMSO = 0.0638, *dwf4* + 10 pM BL > 0.9999, *dwf4* + 50 pM BL = 0.5746, *dwf4* + 100 pM BL = 0.3938, *dwf4* + 250 pM BL = 0.0037. All experiments were repeated independently more than three times with similar results. **d**, Experimental setup for measurements of the root meristem cell production rate (left panel). Six-day-old Col-0 and *dwf4* seedlings were transferred to agar plates supplemented with BL and mock, and after 3 days of growth, elongation of individual roots was recorded for the period of 24 h (E). Subsequently, roots were imaged with confocal microscope, and average mature cell length was calculated (L_{avg}) for each root and used to calculate meristem production rate (P). **e**, Quantification of the root meristem production rate (P) for cortical cells. All individual data points are plotted. Red horizontal bars represent the means and error bars represent s.d. *n* = number of roots analysed. The significant differences between Col-0 and the *dwf4* seedlings treated with BL and Col-0 grown on mock (0) were determined by two-way analysis of variance (ANOVA) and Tukey's multiple comparisons tests. *** $P < 0.001$, ** $P < 0.01$ and * $P < 0.05$. The *P* value versus the Col-0 + DMSO control for Col-0 + 10 pM BL is = 0.0874, Col-0 + 100 pM BL = 0.7849, *dwf4* + DMSO < 0.0001, *dwf4* + 10 pM BL = 0.9623 and *dwf4* + 100 pM BL < 0.0001. The experiment was repeated independently three times with similar results. **f,** Quantification of mature cell length of individual roots used to calculate meristem production rate in (**e**). All individual data points are plotted. Red horizontal bars represent the means and error bars represent s.d.



Extended Data Fig. 6. DWF4 ectopic expression. a, Confocal images of meristems of long and short p35S:DWF4-mCHERRY (DWF4-OE) roots. Six-day-old seedlings were used for imaging. Scale bars, 50 μm. Confocal imaging was performed in three independent experiments. Representative images are shown. b, Two biological replicates of the western blot shown in Fig. 6c. The experiment was repeated independently three times with similar results. **c**, Eight-day-old seedlings expressing DWF4-GFP in *dwf4* mutant background under the control of native or *WEREWOLF (WER)* promoter. Two independent transgenic lines for each construct are compared with wild type (Col-0) and *dwf4*. Plants were grown for 6 days until the point where homozygous *dwf4* plants can be recognized and transferred to fresh agar plates. Root tips were marked (yellow bar), and grown for two additional days. **d**, Confocal images of root meristems of Col-0, *dwf4* and two independent *dwf4* mutant lines expressing DWF4-GFP under

the control of the *DWF4* and *WER* promoters. Six-day-old plants were imaged. Roots were stained with propidium iodide. All transgenic lines were imaged in more than three independent experiments. Representative images are shown. Scale bars, 50 µm. **e-g**, Quantification of cortical meristem cell number (**e**), root meristem diameter (**f**) and cortical meristem cell length of the first 20 cells of individual roots (**g**). All individual data points are plotted. Red horizontal bars represent the means and error bars represent s.d. *n* = number of roots analysed for (**e**) and (**f**) and *n* = number of cells analysed for (**g**). For (**g**) number of individual roots used in experiment are 19, 18, 13, 19, 21 and 20 for Col-0, *dwf4*, line # 18, line # 20, line # 1 and line # 5, respectively. The significant differences between transgenic lines and the Col-0 control were determined by one-way analysis of variance (ANOVA) and Dunnett's multiple comparisons tests. *** $P < 0.001$, ** $P < 0.01$ and * $P < 0.05$. The *P* value versus the Col-0 control for different transgenic lines are as follows: for (**e**) *dwf4* = 0.5724, line # 18 = 0.8377, line # 20 = 0.4041, line # 1 = 0.0308 and line # 5 = 0.0763; for (**f**) *dwf4* < 0.0001, line # 18 = 0.7739, line # 20 = 0.1982, line # 1 < 0.0001 and line # 5 < 0.0001; for (**g**) *dwf4* < 0.0001, line # 18 = 0.0938, line # 20 = 0.0022, line # 1 < 0.0001 and line # 5 < 0.0001. The experiment was repeated independently three times with similar results.



Extended Data Fig. 7. Confined CPD expression can only locally rescue the root phenotype of cpd mutant. a, Six-day-old cpd mutant roots expressing CPD-GFP and CPD-mCHERRY under the control of WEREWOLF (WER) and COBRA-LIKE9 (COBL9) promoters, respectively. All transgenic lines were imaged in more than three independent experiments. Representative images are shown. Scale bars, 100 µm. b, c, Quantification of the root lengths (b) and mature cortical cell lengths (c) of the Col-0, cpd, pWER:CPD-GFP/cpd and pCOBL9:CPD-mCHERRY/cpd lines. All individual data points are plotted. Red horizontal bars represent the means and error bars represent s.d. n = number of roots analysed for (b) and n = number of cells analysed for (c). For (c) number of individual roots used in experiment is 12 for all lines. The significant differences between transgenic lines and the Col-0 control were determined by one-way analysis of variance (ANOVA) and Dunnett's multiple comparisons tests. *** $P < 0.001$, ** $P < 0.01$ and * $P < 0.05$. The P value versus the Col-0 control for different transgenic lines are as follows: for (b) cpd < 0.0001, pWER:CPD-GFP < 0.0001 and pCOBL9:CPD-mCHERRY < 0.0001; for (c) cpd < 0.0001, pWER:CPD-GFP < 0.0001 and pCOBL9:CPD-mCHERRY = 0.049. The experiment was repeated independently three times with similar results. d, Confocal images of root meristems of Col-0, cpd, pWER:CPD-GFP/cpd

and pCOBL9:CPD-mCHERRY/cpd lines. All transgenic lines were imaged in more than three independent experiments. Representative images are shown. Scale bars, 50 μm. e-g, Quantification of cortical meristem cell number (e), cortical meristem cell length of the first 20 cells of individual roots (f) and root meristem diameter (g). All individual data points are plotted. Red horizontal bars represent the means and error bars represent s.d. n = number of roots analysed for (e) and (g) and n = number of cells analysed for (f). For (f) number of individual roots used in experiment is 12 for all lines. The significant differences between transgenic lines and the Col-0 control were determined by one-way analysis of variance (ANOVA) and Dunnett's multiple comparisons tests. *** $P < 0.001$, ** $P < 0.01$ and * $P < 0.05$. The P value versus the Col-0 control for different transgenic lines are as follows: for (**e**) *cpd* = 0.0808, *pWER:CPD-GFP* = 0.0370 and *pCOBL9:CPD-mCHERRY* = 0.0323; for (**f**) *cpd* < 0.0001, *pWER:CPD-GFP* = 0.011 and *pCOBL9:CPD-mCHERRY* = < 0.0001; for (**g**) *cpd* < 0.0001, *pWER:CPD-GFP* = 0.2712 and *pCOBL9:CPD-mCHERRY* = < 0.0001. The experiment was repeated independently three times with similar results.

Supplementary Video 1. Long-term confocal imaging of 6-day-old *pDWF4WF4-GFP*/*dwf4* roots by vertical-stage microscopy.

Supplementary Video 2. Long-term confocal imaging of 6-day-old *pROT3:ROT3-GFP/rot3* roots by vertical-stage microscopy.

Supplementary Video 3. Long-term confocal imaging of 6-day-old *pCPD:CPD-GFP/cpd* roots by vertical-stage microscopy.

Supplementary Video 4. Long-term confocal imaging of 6-day-old *pBES1:BES1-GFP* roots by vertical-stage microscopy.