

Supplementary Fig. 1. Accessions used in this study and representative root system images of selected accessions.

(a) Geographical distribution of natural *Arabidopsis thaliana* accessions used in this study. The distribution of accessions is visualized by R package “worldmap”.

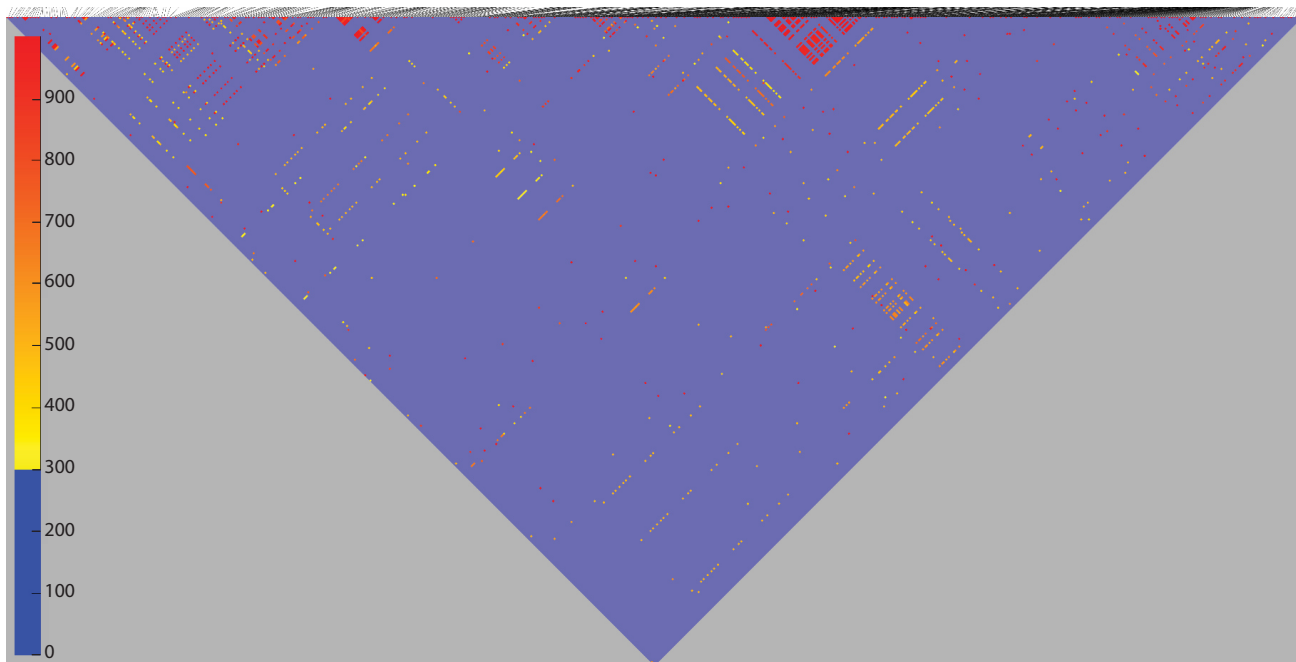
(b) 10-day old accessions grown in 3D agar cylinders. Scale bars, 1 cm.

(c) 20-day old accessions grown in soil. Scale bars, 5 cm.

(b)-(c) Representative images are shown. Experiments were repeated at least three times.



AT2G19500.1

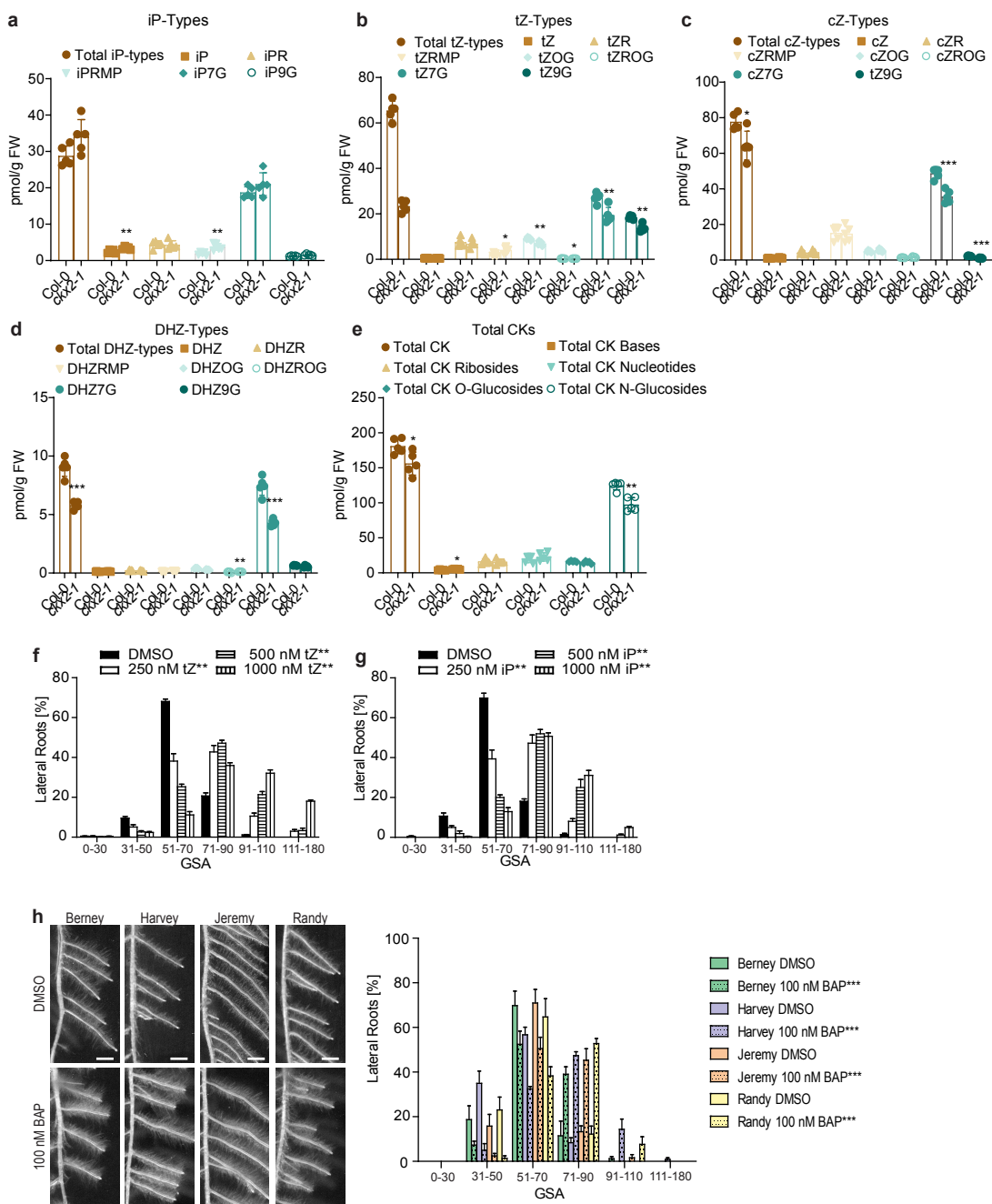


Supplementary Fig. 2. Calculation of linkage disequilibrium by pairwise comparison of 500 SNPs.

r^2 value is scaled and color-coded (blue to red) from 0 to 1 (low to high association).

Underlying code can be found at github

<https://github.com/timeu/PyGWAS/blob/master/pygwas/core/genotype.py#L59>.



Supplementary Fig. 3. Influence of cytokinin on GSA of lateral roots.

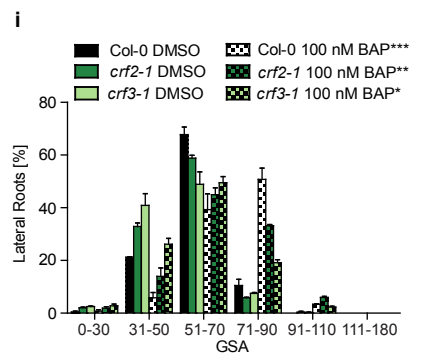
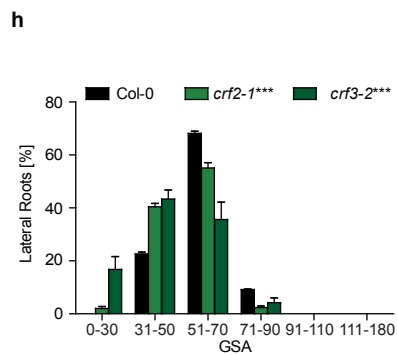
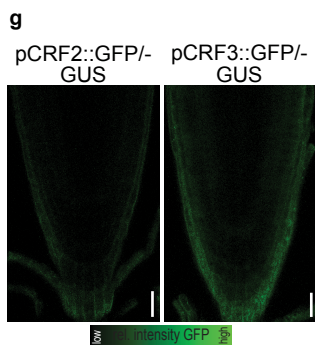
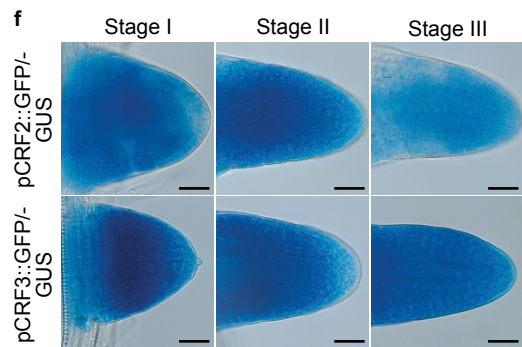
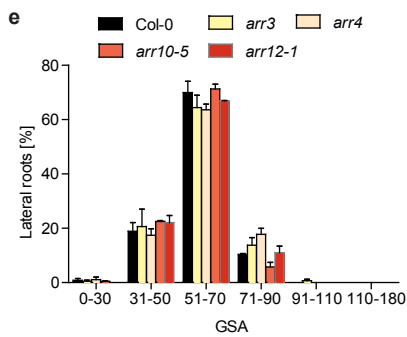
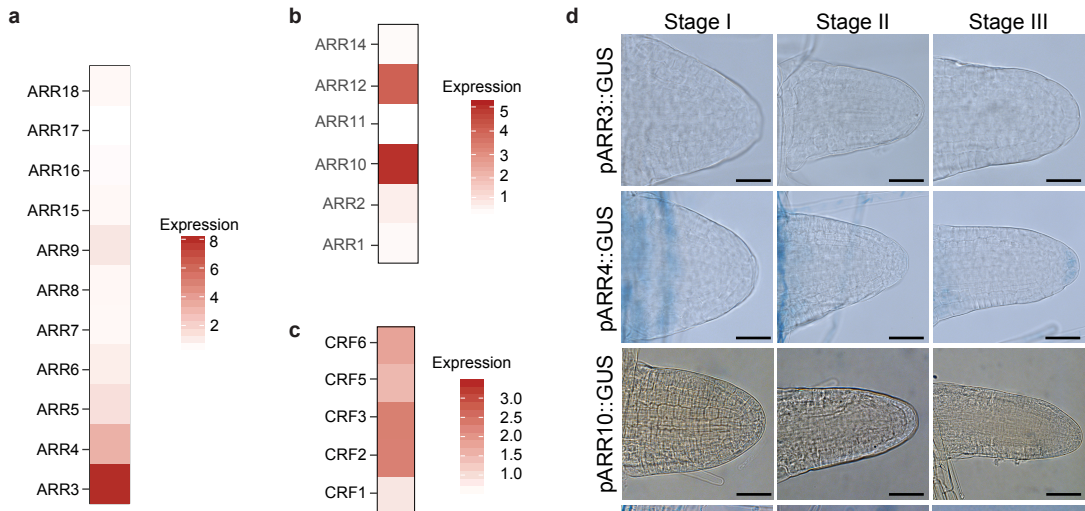
(a)-(e) Quantification of different CK forms (nucleotides (precursors), ribosides (transported forms), free bases (active forms), and O-/N-glucosides (reversible/irreversible inactivated storage forms) in *Col-0* wild type and *cks2-1* mutant roots. (a) iP-Types, (b) tZ-Types, (c) cZ-Types, (d) DHZ-Types, (e) Total CKs.

(f)-(g), GSA distributions of DMSO and (f) trans-zeatin (tZ)-treated or (g) isopentenyladenine (iP)-treated *Col-0* wild type seedlings.

(h) Representative images and GSA distributions of four different untreated and BA-treated winter oilseed rape (*Brassica napus* L.) genotypes.

(a)-(e) One-way ANOVA analysis P-values: * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$. Mean \pm SD, $n = 5$ extractions.

(f)-(h) Kolmogorov-Smirnov test P-values: * $P < 0.05$, ** $P < 0.01$, *** $P > 0.001$ (compared to DMSO or *Col-0*, respectively). Mean \pm SEM, for *A. thaliana*: $n = 5$ plates (16 seedlings with 80-160 LR per plate), for oilseed rape: $n = 3$ seedlings with 25-50 LR per seedling). Experiments were repeated at least three times.



Supplementary Fig. 4. Role of Arabidopsis response regulators (ARRs) and cytokinin response factors (CRFs) in the GSA establishment in LRs.

(a) Expression of type-A ARR in lateral roots. Data from²⁵.

(b) Expression of type-B ARR in lateral roots. Data from²⁵.

(c) Expression of CRFs in lateral roots. Data from²⁵.

(d) GUS staining of pARR3::GUS, pARR4::GUS, pARR10::GUS and pARR12::GUS. Scale bars, 25 μ m.

(e) GSA distribution of *Col-0* wild-type, *arr3*, *arr4*, *arr10-5* and *arr12-1* mutants. Kolmogorov-Smirnov test. Mean \pm SEM, n = 5 plates (16 seedlings with 25-120 LRs per plate).

(f) Representative images after GUS staining of pCRF2::GFP-GUS and pCRF3::GFP-GUS in stage I-III LRs. Scale bars, 10 μ m.

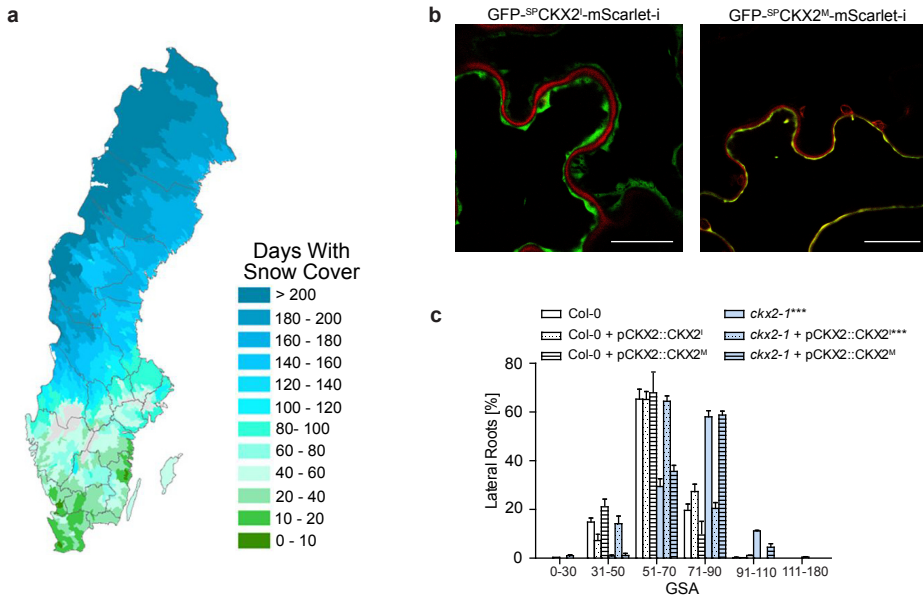
(g) Representative images of pCRF2::GFP-GUS and pCRF3::GFP-GUS in the main root tip. Scale bars, 25 μ m.

(h) GSA distribution of *Col-0* wild type and *crf* single mutants.

(i) GSA distribution of DMSO and BAP treated *Col-0* wild type, *crf2-1* and *crf3-1* single mutants.

(h) – (i) Kolmogorov-Smirnov test P-values: * P < 0.05, ** P < 0.01, *** P > 0.001 (compared to DMSO or *Col-0*). Mean \pm SEM, n = 5 plates (16 seedlings with 20-180 LRs per plate).

(d)-(i) Experiments were repeated three times.



Supplementary Figure 5. Snow cover in Sweden and characterization of CKX2^l and CKX2^M.

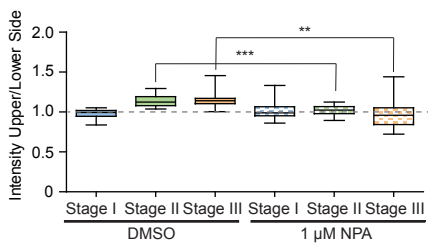
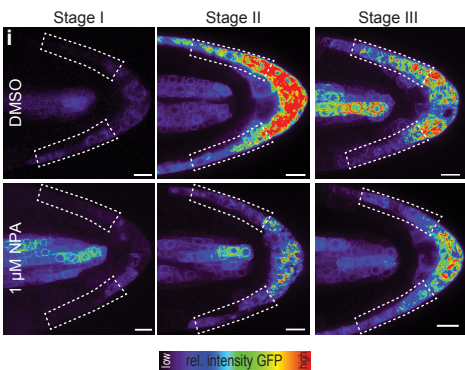
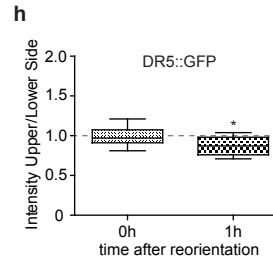
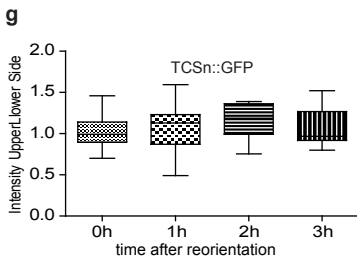
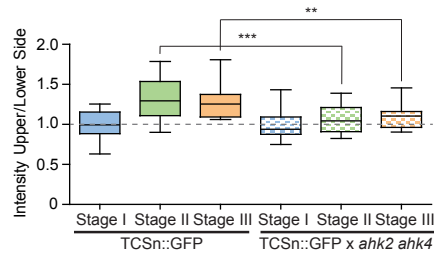
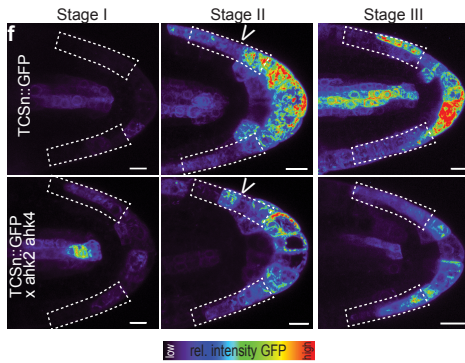
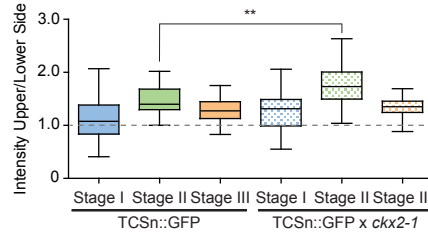
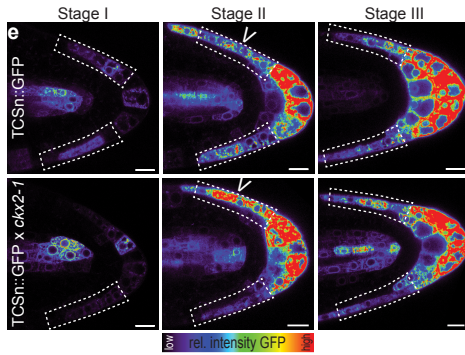
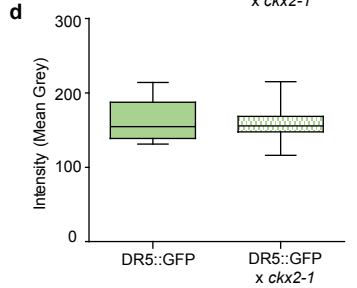
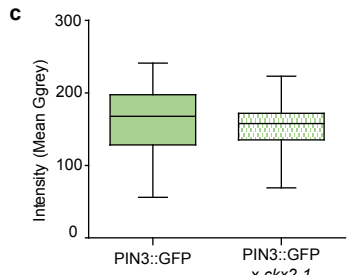
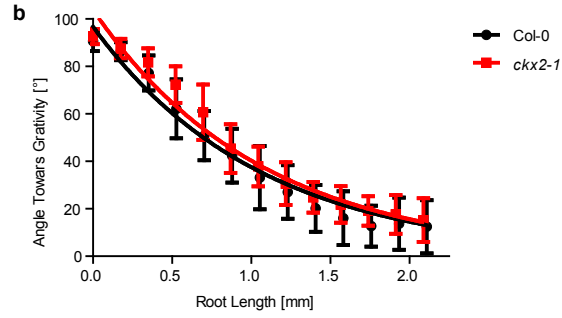
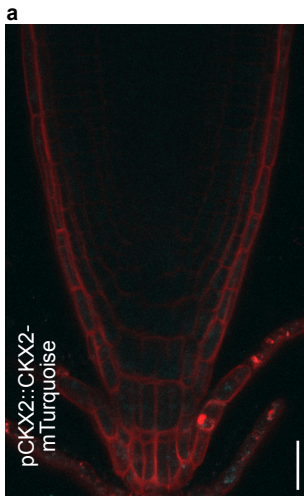
Supplementary Fig. 5. Snow cover in Sweden and characterization of CKX2^l and CKX2^M.

(a) Average number of days with snow cover in Sweden between 1961-1990. Source: <https://bit.ly/2UmLAeT>

(b) Localization of GFP-^{SP}CKX2^l-mScarlet and GFP-^{SP}CKX2^M-mScarlet. Tobacco leaves were infiltrated with *Agrobacterium tumefaciens* containing constructs and the expression of CKX2 fluorescent protein was visualized by confocal laser scanning microscopy three days after infiltration. Scale bar, 25 μ m. Representative images are shown.

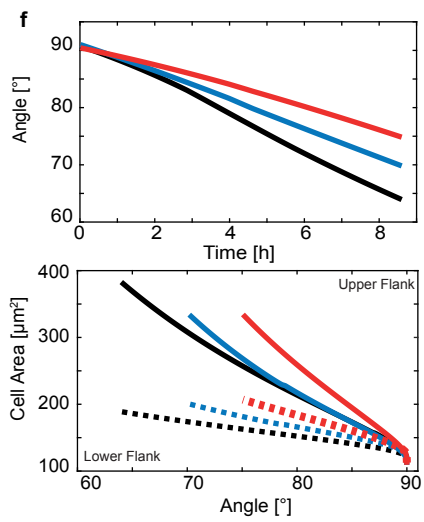
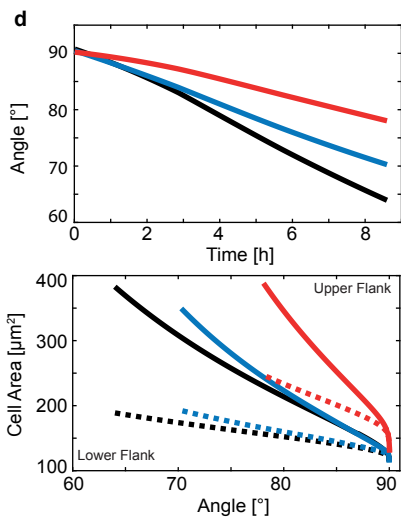
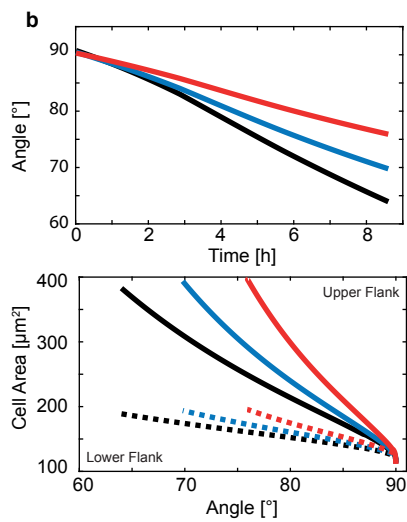
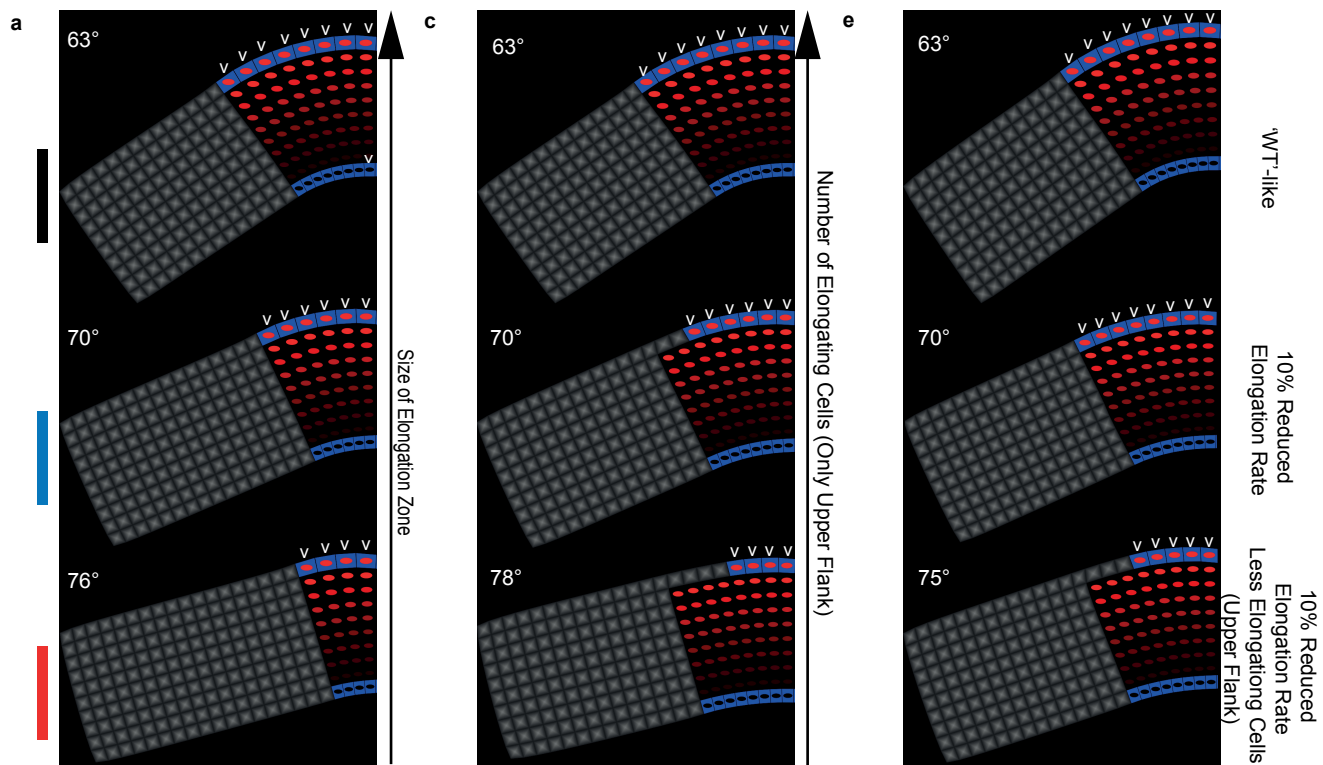
(c) GSA distributions of *Col-0* and *cks2-1* as well as CKX2^l and CKX2^M expressing lines in both backgrounds. Kolmogorov-Smirnov test P-value: *** P < 0.001 (compared to *Col-0*). Mean \pm SEM, n = 5 plates (16 seedlings with 80-160 LRs per plate).

(b)-(c) Experiments were repeated at least three times.



Supplementary Fig. 6. Localization of CKX2 in the main root tip and characterization of cytokinin responses in *ckx2-1* mutants.

- (a) Representative images of pCKX2::CKX2-mTurquoise in the main root. Propidium iodide (PI) was used for counterstaining. Scale bar, 25 μ m.
- (b) Gravitropic response of dark grown *Col-0* and *ckx2-1*. Mean \pm SD, n = 10-15 individual roots.
- (c)-(d) Signal quantification of (b) pPIN3::PIN3-GFP and (c) DR5::GFP in stage II LR. s.
- (e)-(f) Representative images and quantifications of (a) TCSn::GFP in *Col-0* wild type and *ckx2-1*, (b) TCSn::GFP in *Col-0* wild type and *ahk2 ahk4* in stage I – III LR. s. Scale bar, 10 μ m.
- (g) Signal quantification of TCSn::GFP in the main root after gravity stimulation.
- (h) Signal quantification of DR5::GFP in the main root after gravity stimulation (positive control for (g)).
- (i) Representative images and quantifications of TCSn::GFP in *Col-0* wild type after treatment with DMSO or 1 μ M NPA for 24h in stage I – III LR. s. Scale bar, 10 μ m.
- (c)-(d), (e)-(i) One-way ANOVA P-values: * P < 0.05, ** P < 0.01, *** P < 0.001. Horizontal lines show the medians; box limits indicate the 25th and 75th percentiles; whiskers extend to the min and max values. n = 10-40 individual LR. s or main roots.
- (a)-(i) Experiments were repeated at least three times.



Supplementary Figure 7. Simulations of dynamic computer model of lateral root predict an inverse relation between set-point angle and the number of cells in elongation zone or the size of elongation zone.

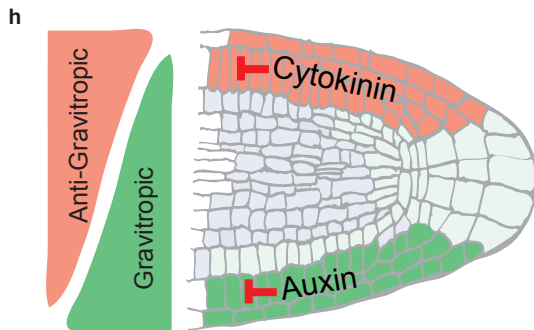
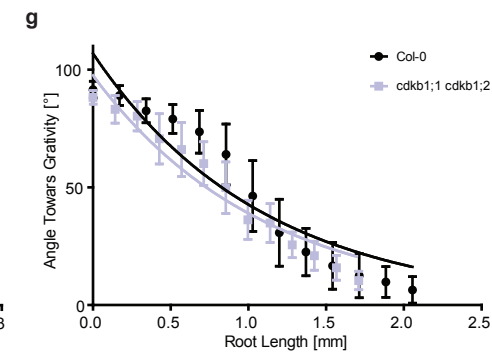
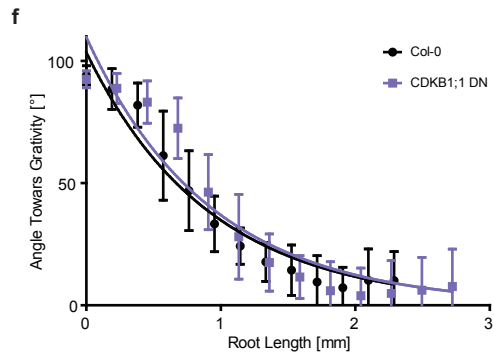
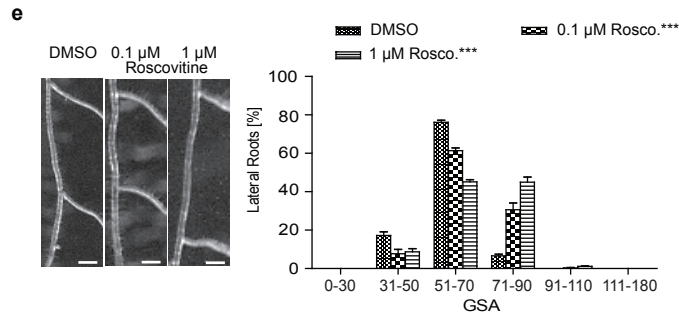
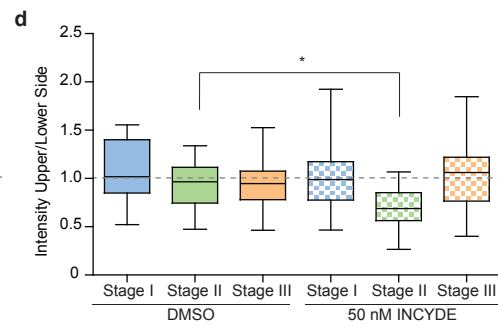
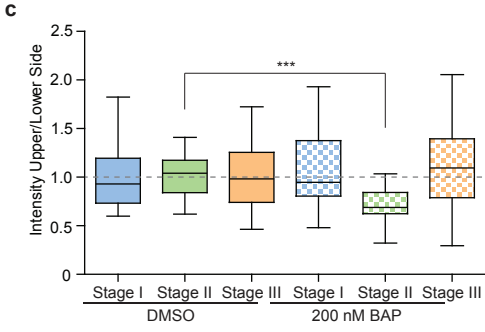
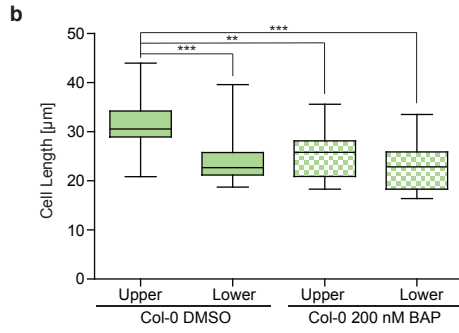
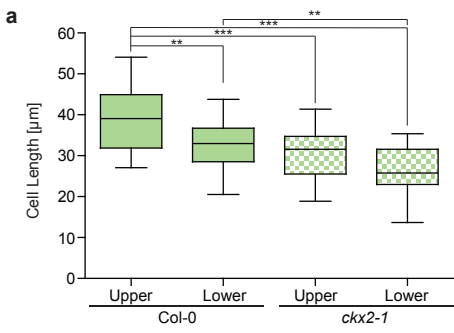
(a)-(b) A positive impact of the size of elongation zone on the GSA angle. The longer the elongation zone (white arrow heads) the larger the GSA angle is.

(c)-(d) The number of cells on the upper flank has strong negative effect on the GSA angle. Also, model predicts that the fold difference between the size of elongating cell on the upper versus lower LR root flanks may decrease with strongly reduced number of cells, largely due to mechanical constraints present on the upper root flank.

(e)-(f) Upper panel, corresponds Fig. 7b. Middle panel, 10% decrease in elongation rate only on the upper root flank (white arrow heads). Lower panel, combination of 10% decrease in elongation rate and number of cells (shorter meristem) only on the upper root flank. Each simulation represents LR status after 9h of dynamic elongation.

(b), (d), (f) Upper panel: Time evolution of set-point angle corresponding to respective scenarios. Lower panel: Predicted GSA angle plotted against the predicted cell size.

Colours at the left side (a), (c), (e) matches simulations in (b), (d), (f). Simulations represent LR status after 8h of dynamic elongation. Other symbols are as in Figure 7.



Supplementary Figure 8. Cytokinin-dependent interference with cell elongation and division rates defines angular growth of lateral roots.

(a)-(b) Quantification of first two elongated cells in the upper and lower flank of stage II lateral roots of (a) *Col-0* and *ckx2-1* and (b) after treatment with DMSO or 200 nM BAP for 24h.

(c)-(d) Quantification of pCycB1;1::GUS after treatment with (c) DMSO or 200 nM BAP or (d) DMSO or 50 nM INCYDE for 24h.

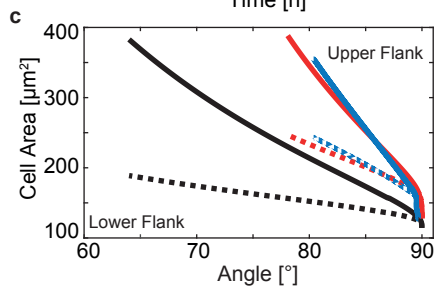
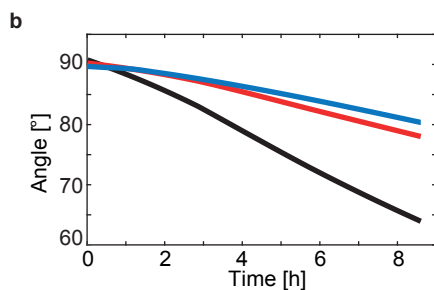
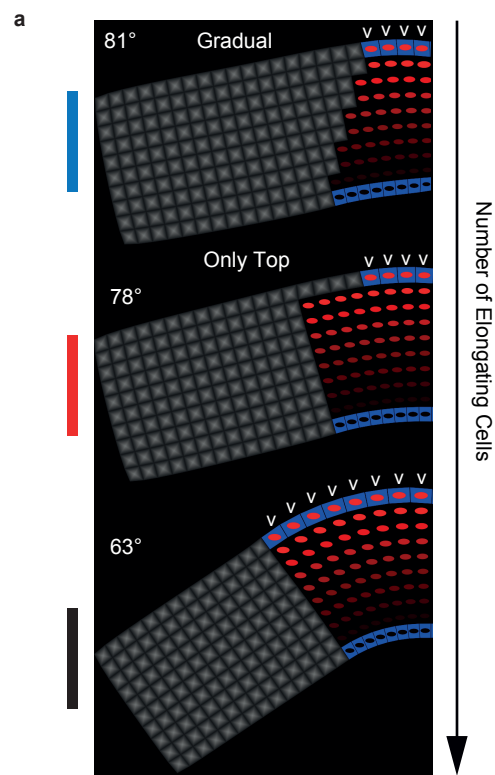
(a)-(d) One-way ANOVA P-values: * $P < 0.05$, *** $P < 0.001$. Horizontal lines show the medians; box limits indicate the 25th and 75th percentiles; whiskers extend to the min and max values. (a)-(b) $n = 10-15$ individual LRs, (c)-(d) $n = 15-40$ individual LRs.

(e) Representative images and GSA distributions of Roscovitine treated *Col-0* wild-type seedlings. Kolmogorov-Smirnov test P-values: *** $P < 0.001$ (compared to DMSO solvent control). Mean \pm SEM, $n = 5$ plates (16 seedlings with 90-130 LRs per plate). Scale bars, 2 mm.

(f)-(g) Gravitropic growth rates to quantitatively assess gravitropic response of dark grown (f) *Col-0* and *CDKB1;1 DN* as well as (g) *Col-0* and *cdkb1;1 cdkb1;2*. Mean \pm SD, $n = 10-15$ individual roots.

(a)-(g) Experiments were repeated at least three times.

(h) Schematic model depicts spatially defined gravitropic and anti-gravitropic hormonal cues at opposing organ flanks. Cytokinin signalling functions as an anti-gravitropic growth regulator at the upper side and thereby counterbalances auxin-dependent gravitropic growth of lateral roots.



Supplementary Figure 9. Simulations of dynamic computer model addressing the gradual decrease of cell numbers along a lateral root organ.

(a) Simulated reduction of elongating cells, which are either restricted to the upper part (red) or gradually extent from the bottom towards the top (blue), induce reduced curvature compared to the control scenario (black).

(b) Time evolution of set-point angle corresponding to respective scenarios.

(c) Predicted GSA angle plotted against the predicted cell size. Dashed and filled lines depict lower and upper root flank, respectively. Colours in (b) and (c) correspond to (a). Simulations represent LR status after 8h of dynamic elongation.