nature portfolio

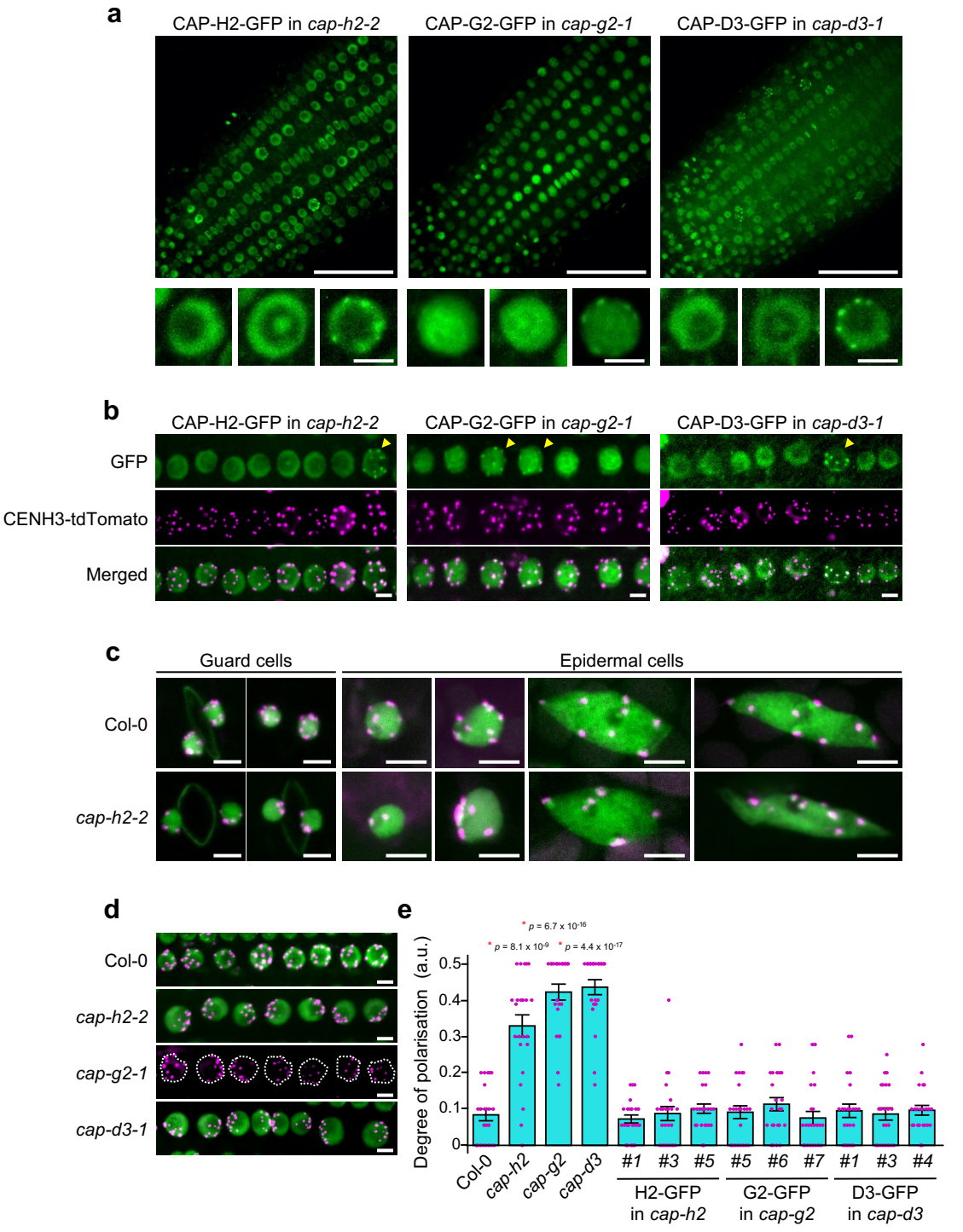
https://doi.org/10.1038/s41477-022-01200-3

Supplementary information

Two-step regulation of centromere distribution by condensin II and the nuclear envelope proteins

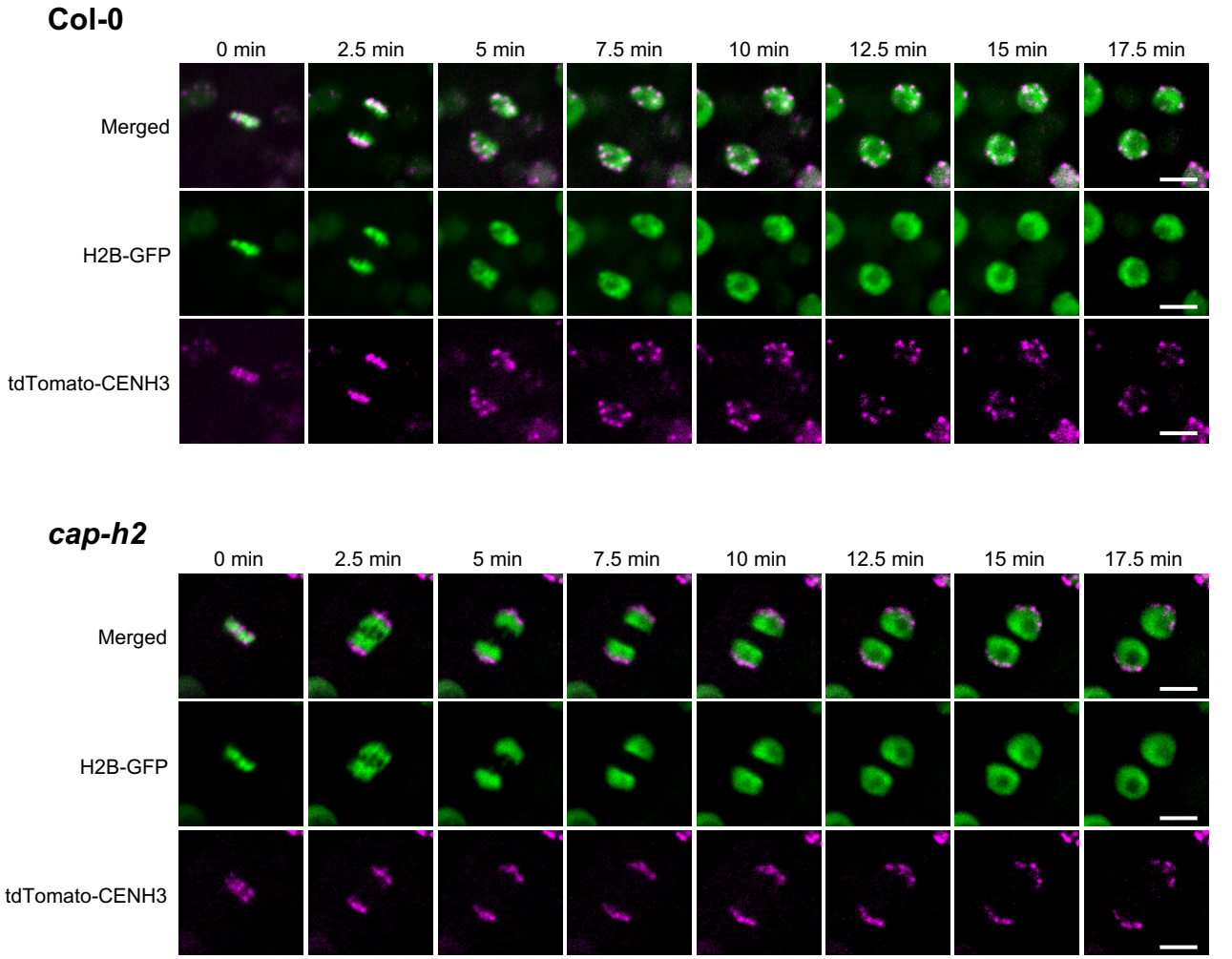
In the format provided by the authors and unedited

Supplementary Fig.1



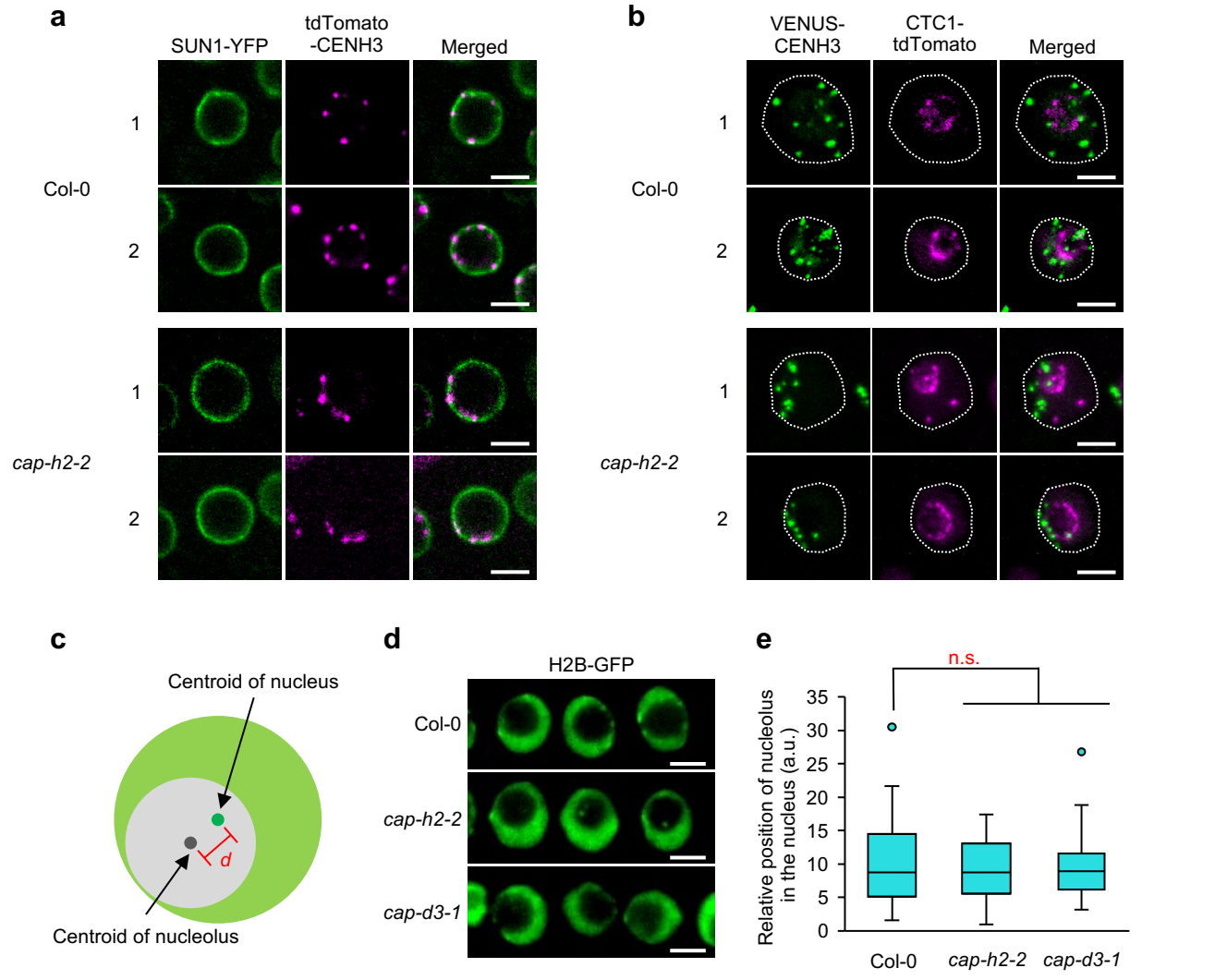
Supplementary Figure 1. Demonstration of the requirement of condensin II for scattering centromeres
a, Expression patterns of condensin II in the root meristems. Condensin II-specific subunits, CAP-H2, CAP-G2, and CAP-D3, were fluorescently visualised using *pCAP-H2::CAP-H2-GFP*, *pCAP-G2::CAP-G2-GFP* and *pCAP-D3::CAP-D3-GFP* in the respective mutants. The lower panels show the localisation patterns of condensin II in the nucleus; nucleoplasm only, nucleoplasm and central part of the nucleolus, whole nucleus including the nucleolus, and spot-like localisation in the nucleoplasm. The images are shown as maximum *z*-projections (upper panels) and optical sections (lower panels). Bars = 50 μm (upper panels) and 5 μm (lower panels). **b**, Simultaneous observation of condensin II and CENH3 fluorescently visualised with GFP-fused condensin II subunits (green) and *p35S::tdTomato-CENH3* (magenta) in the nuclei of the root meristem in the respective mutants. Arrowheads indicate nuclei with spot-like localisation of condensin II. The images are shown as maximum *z*-projections. Bars = 5 μm. **c**, Distribution of centromeres visualised with *p35S::tdTomato-CENH3* in interphase nuclei in the guard cells and leaf epidermal cells of *cap-h2-2*. The nuclei were visualised using *pRPS5a::H2B-GFP*. The images are shown as maximum *z*-projections. Bars = 5 μm. **d**, Distribution of centromeres visualised with *p35S::tdTomato-CENH3* in interphase nuclei in the root meristem of the condensin II-specific subunit mutants. The nuclei were visualised using *pRPS5a::H2B-GFP*. The images are shown as maximum *z*-projections. Bars = 5 μm. **e**, Quantitative analysis of the degree of polarisation in centromere distribution in interphase nuclei in the root meristem of condensin II mutants and complementation lines of each mutant. The graph shows average ± s.e.m., n = 23 nuclei, *P < 0.001, two- sided Student's t-test.

Supplementary Fig.2



Supplementary Figure 2. Dynamics of centromeres in wild-type and *cap-h2*
Dynamics of centromeres visualised in magenta using *p35S::tdTomato-CENH3* from the metaphase to the early G1 phase. Nuclei are visualised in green using *pRPS5a::H2B-GFP*. This figure shows the split images of the composite images shown in Figure 2c. Bars = 5 µm.

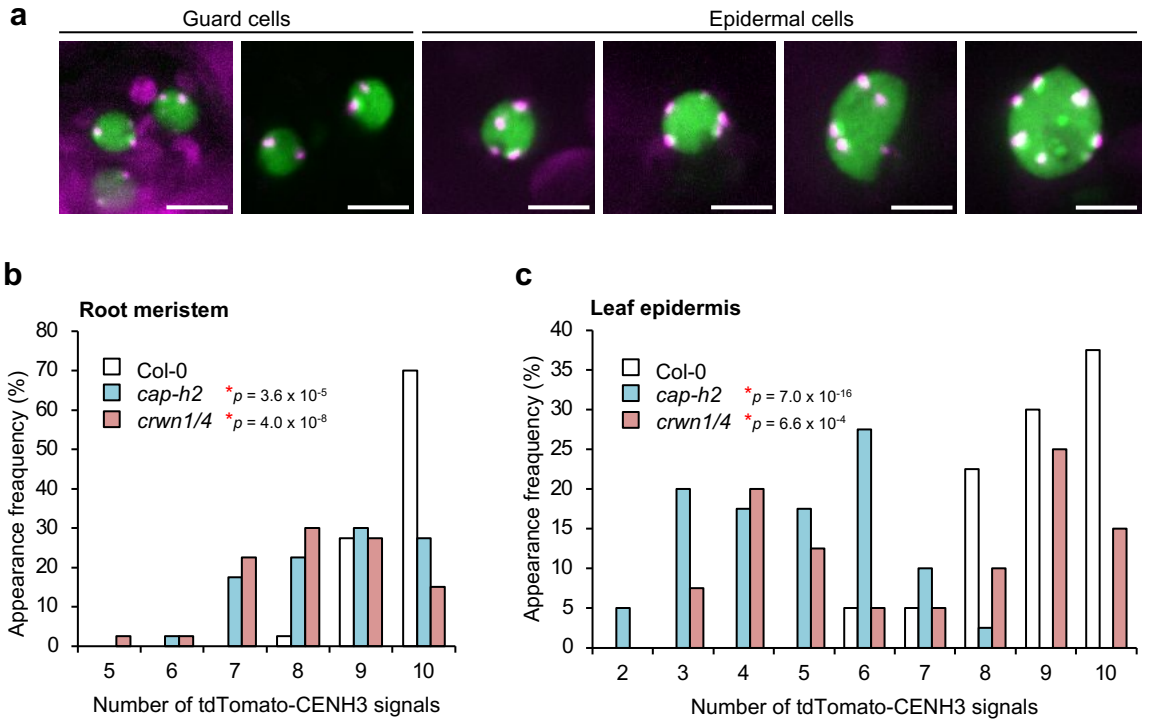
Supplementary Fig.3



Supplementary Figure 3. Condensin II is not involved in the peripheral localisation of centromeres and the distribution of telomeres and nucleoli

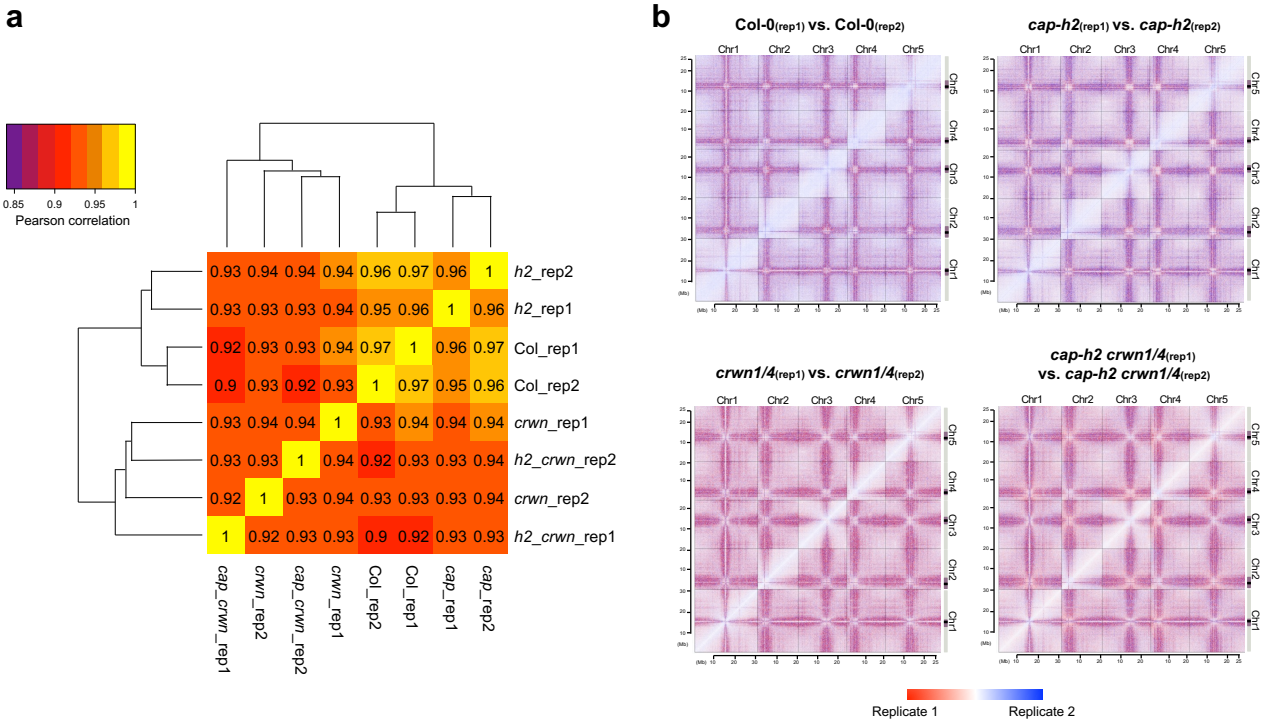
a, Simultaneous observation of centromeres and nuclear periphery fluorescently visualised with *p35S::tdTomato-CENH3* (magenta) and *p35S::SUN1-YFP* (green), respectively, in interphase nuclei of the root meristem. Roots of 7-day-old seedlings were also observed. The optical sections closest to the centre of the nucleus in two different cells are shown. Bars = 5 μm. **b**, Simultaneous observation of centromeres and telomeres fluorescently visualised with *p35S::VENUS-CENH3* (green) and *pRPS5a::CTC1-tdTomato* (magenta), respectively, in interphase nuclei of the root meristem. Roots of 7-day-old seedlings were observed; the periphery of nuclei is indicated by a white dotted line. The images are shown as maximum *z*-projections. Bars = 5 μm. **c,** Evaluation of the relative position of the nucleolus in the nucleus. The distance between the centroid of the nucleus and that of the nucleolus was measured and normalised to the ratio of the nucleolus area to the nucleus area. **d**, Representative images of nuclei and nucleoli in the epidermis of the root meristem of wild type and condensin II mutant plants. The nucleolus was defined as the nuclear region in which no *pRPS5a::H2B-GFP* signal (green) was detected. The optical section closest to the centre of the nucleus is shown. Bars = 5 μm. **e**, Relative position of the nucleolus in the nucleus in wild type and condensin II mutant plants. The first and third quartiles with the median are shown as boxes with cross bars; lower and upper whiskers represent the entire range of data points; dots indicate outliers. Two-sided Student's *t*-test was performed. *n* = 40 nuclei from at least three independent plants. Experiments, **a, b** and **d**, were at least independently repeated twice with similar results.

Supplementary Fig.4



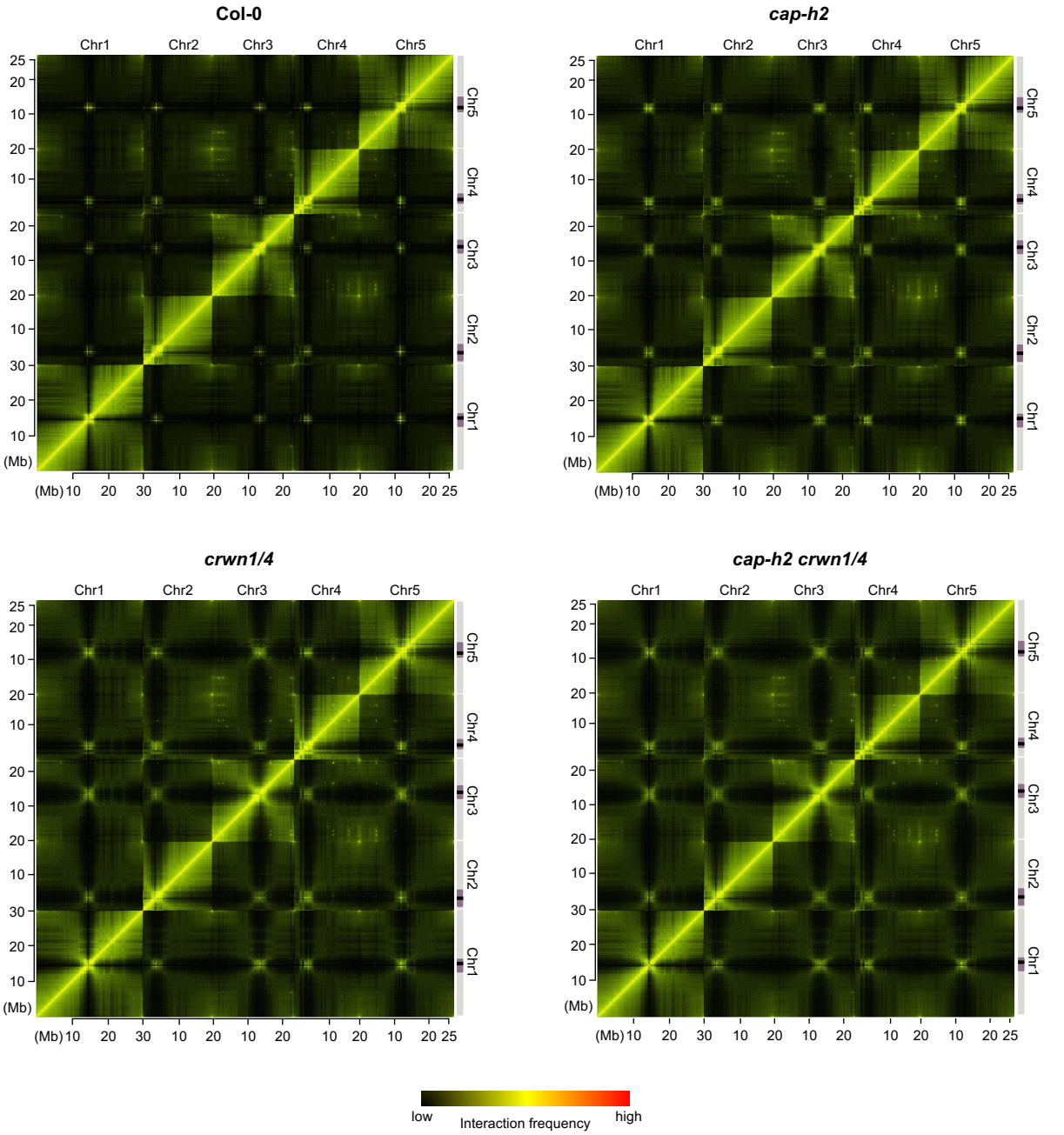
Supplementary Figure 4. Mutations in condensin II and CRWNs reduce the number of separated centromere signals
a, Distribution of centromeres visualised with *p35S::tdTomato-CENH3* in the interphase nuclei of guard cells and leaf epidermal cells in *crwn1/4*. The nuclei were visualised using *pRPS5a::H2B-GFP*. True leaves from 10-day-old seedlings were observed. The images are shown as maximum *z*-projections. Bars = 5 μm. **b,c** The number of separated centromere signals per nucleus in the root meristem (**b**) and leaf epidermis (except for guard cells) (**c**) was counted. The frequency distribution of the number of centromere signals in each mutant was statistically compared with that in Col-0 using a two-sided Fisher's exact test (*$P < 0.001$, $n = 40$ nuclei from at least three independent plants). Roots of 7-day-old seedlings and true leaves of 10-day-old seedlings were observed.

Supplementary Fig.5



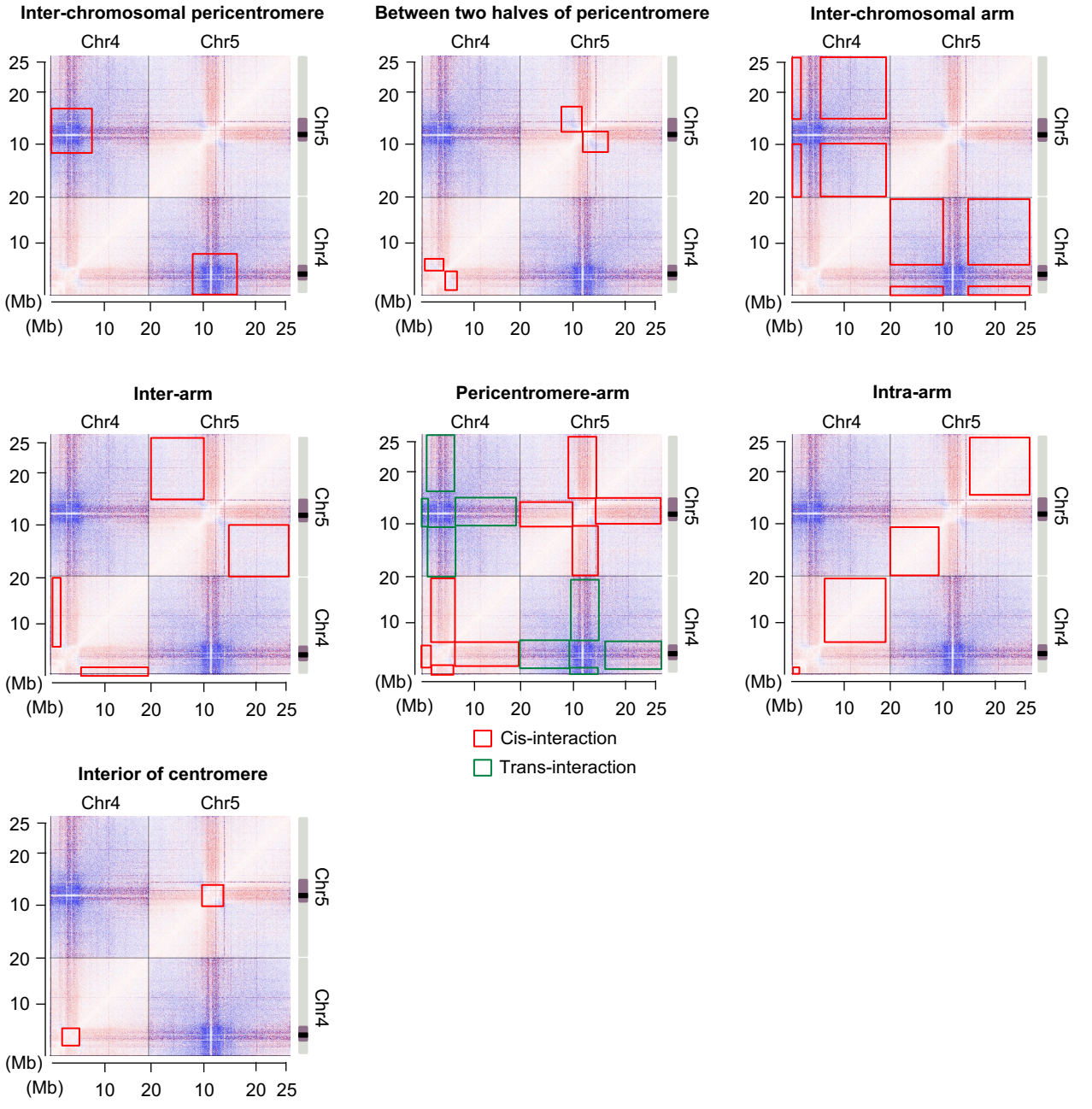
Supplementary Figure 5. Validation of Hi-C data.
a, Comparison of genome-wide Hi-C maps from various samples (bin size: 50 kb). **b**, Enrichment of interaction frequencies, obtained by calculating the relative difference between Hi-C maps of the two respective biological replicates (bin size: 50 kb).

Supplementary Fig.6



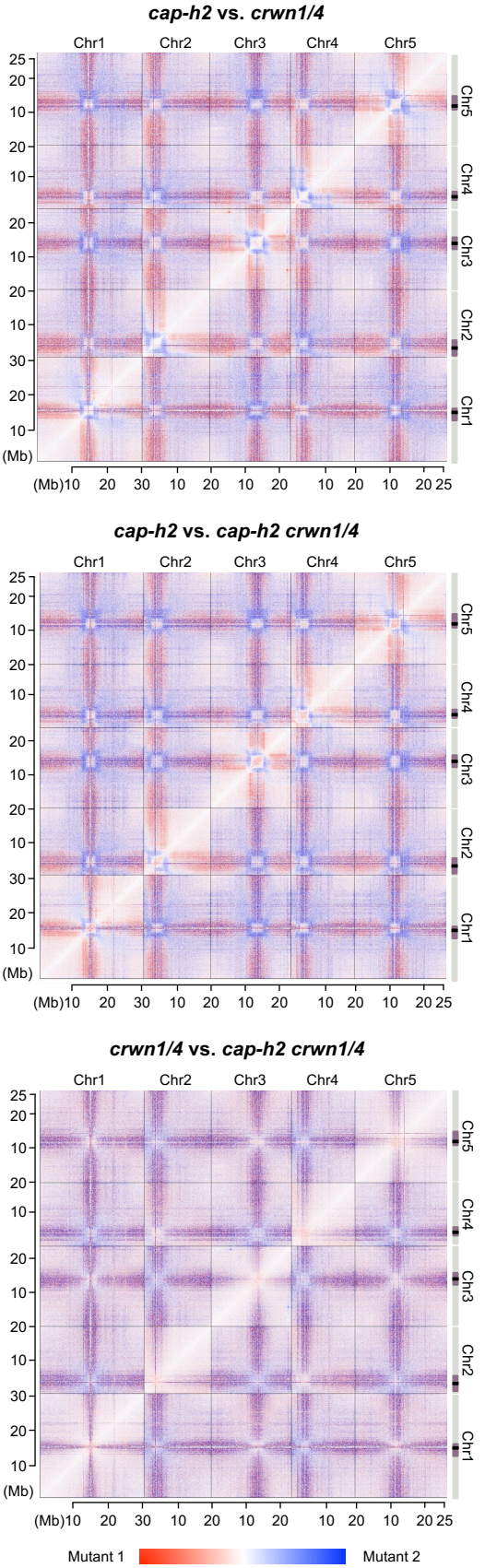
Supplementary Figure 6. Visualisation of Hi-C interaction frequency
Merged data were used. Bin size = 50 kb.

Supplementary Fig.7



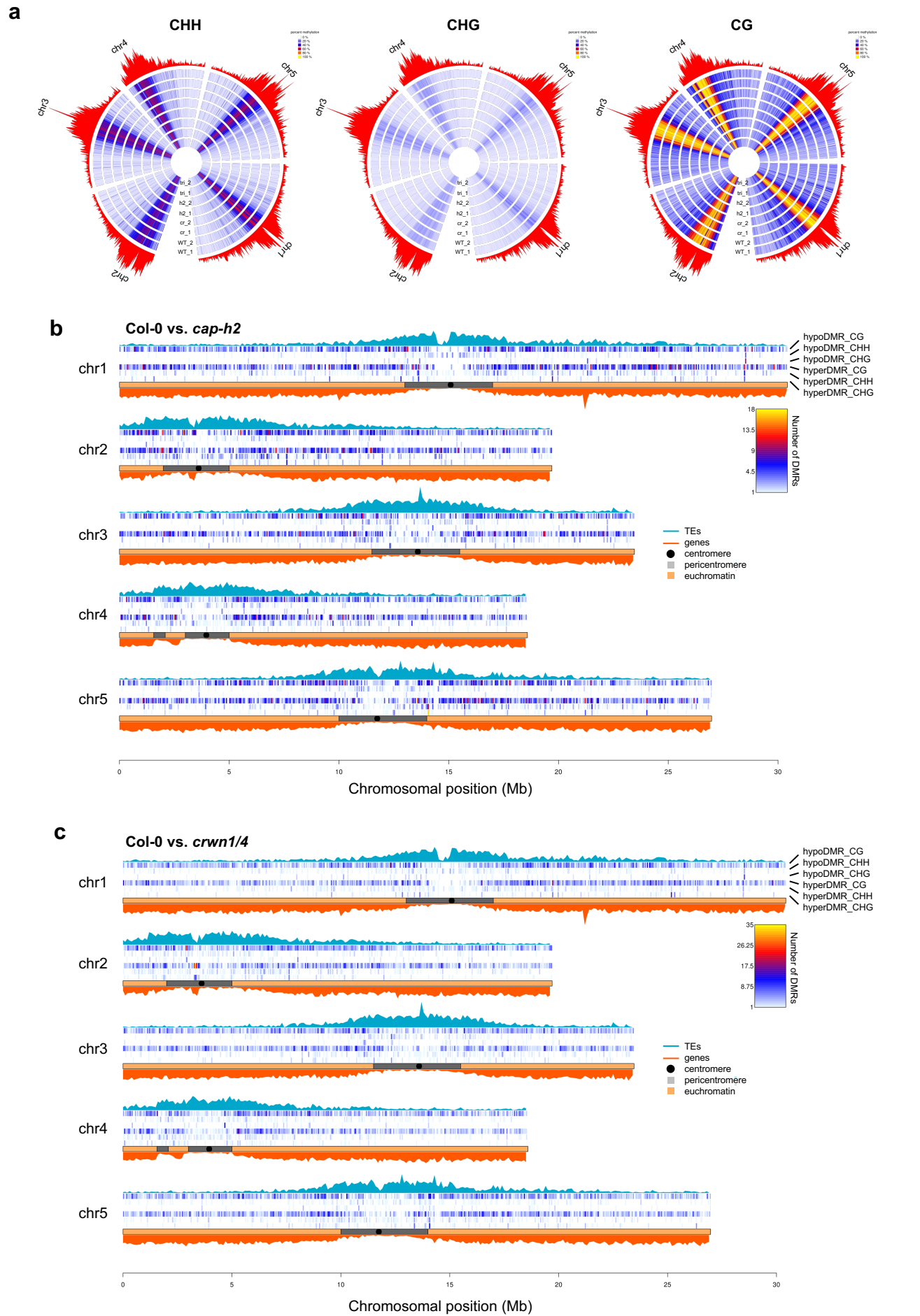
Supplementary Figure 7. Regions of chromatin interactions described in the manuscript.
The regions pointing to each type of chromatin interaction we described are shown. An example of each type is shown with a magnified image of the chromatin interaction between chromosomes 4 and 5 of the relative difference between Col-0 and *cap-h2-2*.

Supplementary Fig.8

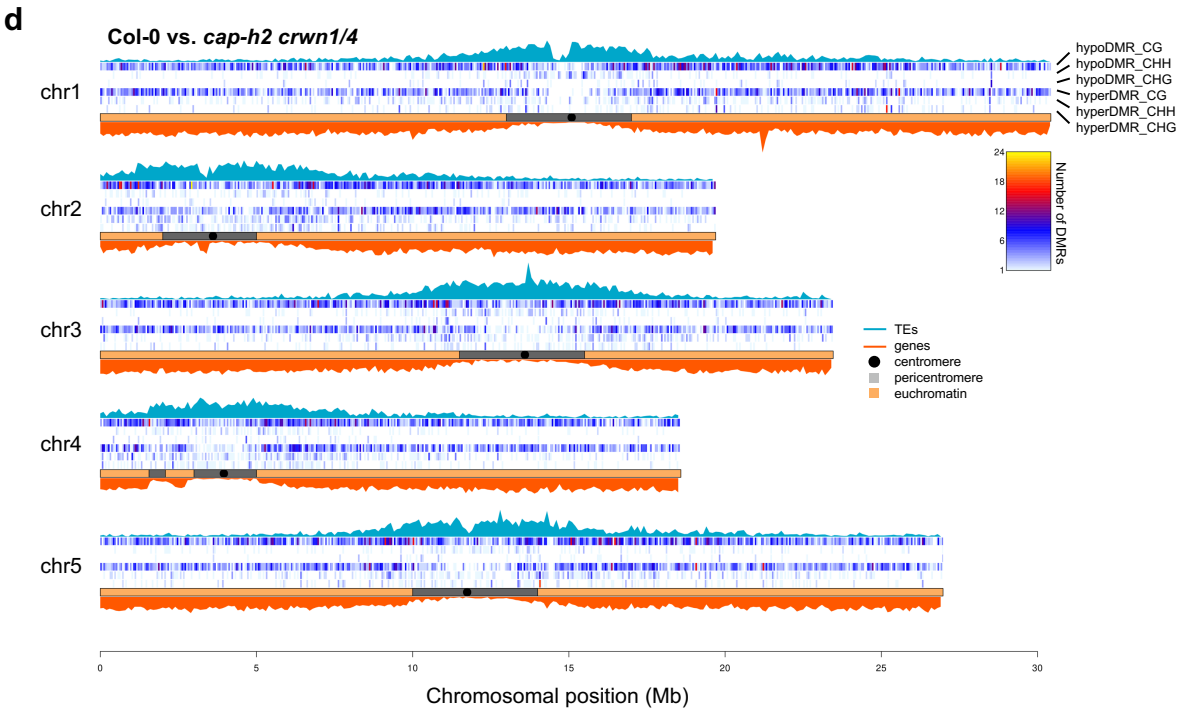


Supplementary Figure 8. Comparison of chromatin organization between the mutants of condensin II and CRWNs
Enrichment of interaction frequency was obtained by calculating the relative difference between the two interactomes of the indicated lines. Bin size = 50 kb.

Supplementary Fig.9

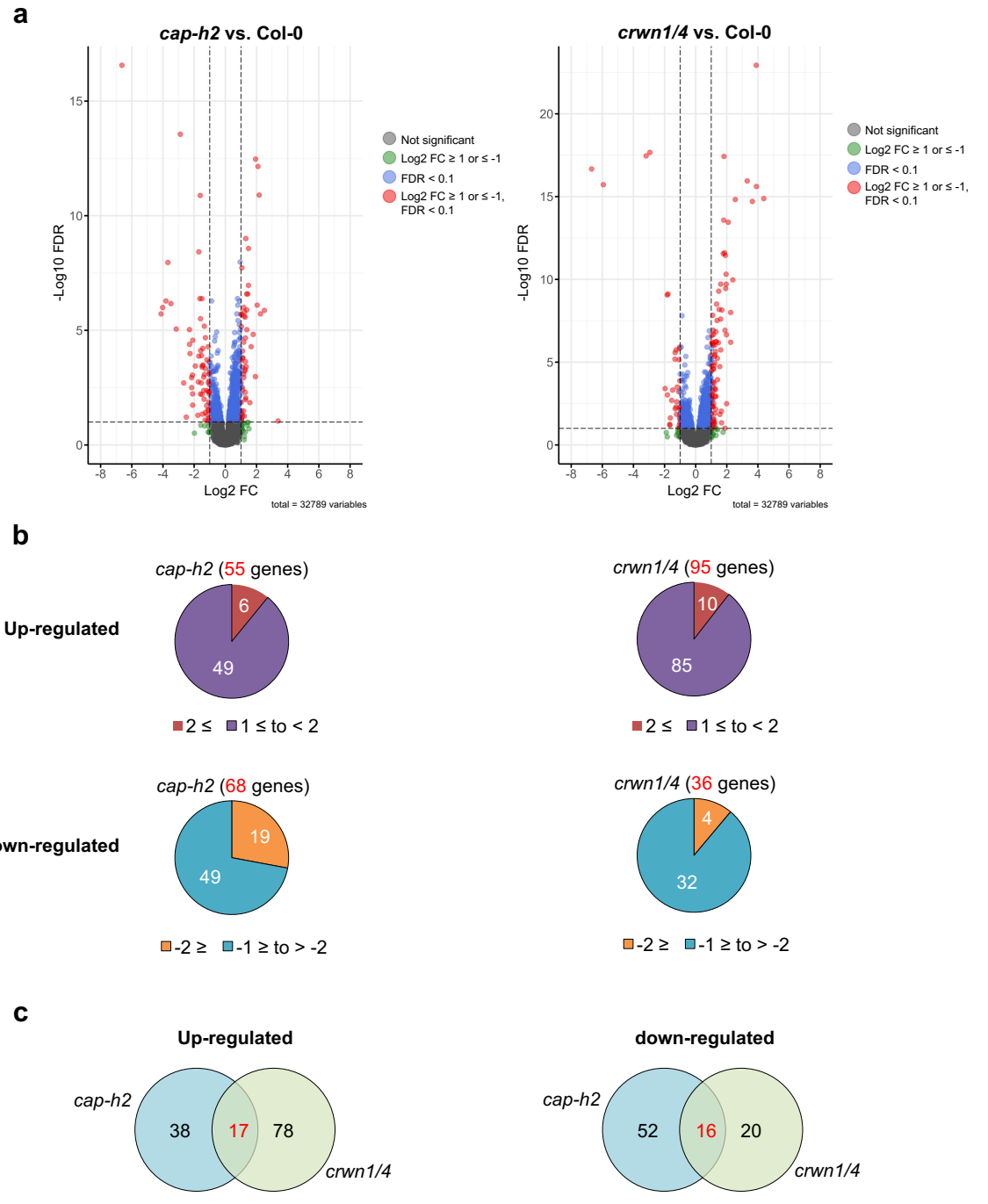


Supplementary Fig.9 continued



Supplementary Figure 9. Genome-wide DNA methylation in the mutants showing abnormal centromere distribution
a, Circos plot for cytosine methylation levels in the CHG, CHH, and CG contexts. Red bar plots indicate the densities of transposable elements within 50 kb genomic bins. Two replicates for each genotype are shown. **b–d,** Chromosomal plot showing the number of differentially methylated bins along the five *A. thaliana* chromosomes in *cap-h2-2* (**b**), *crwn1/4* (**c**), and *cap-h2-2 crwn1/4* (**d**). The colour code refers to the number of differentially methylated 100 bp DNA bins within 50 kb genomic regions. Differentially methylated regions (DMRs) were calculated using merged data from two replicates for each genotype. Slight changes in methylation levels were detected throughout the chromosomes in the mutants, but there was no positional specificity of hypo- and hyper-methylation in all contexts.

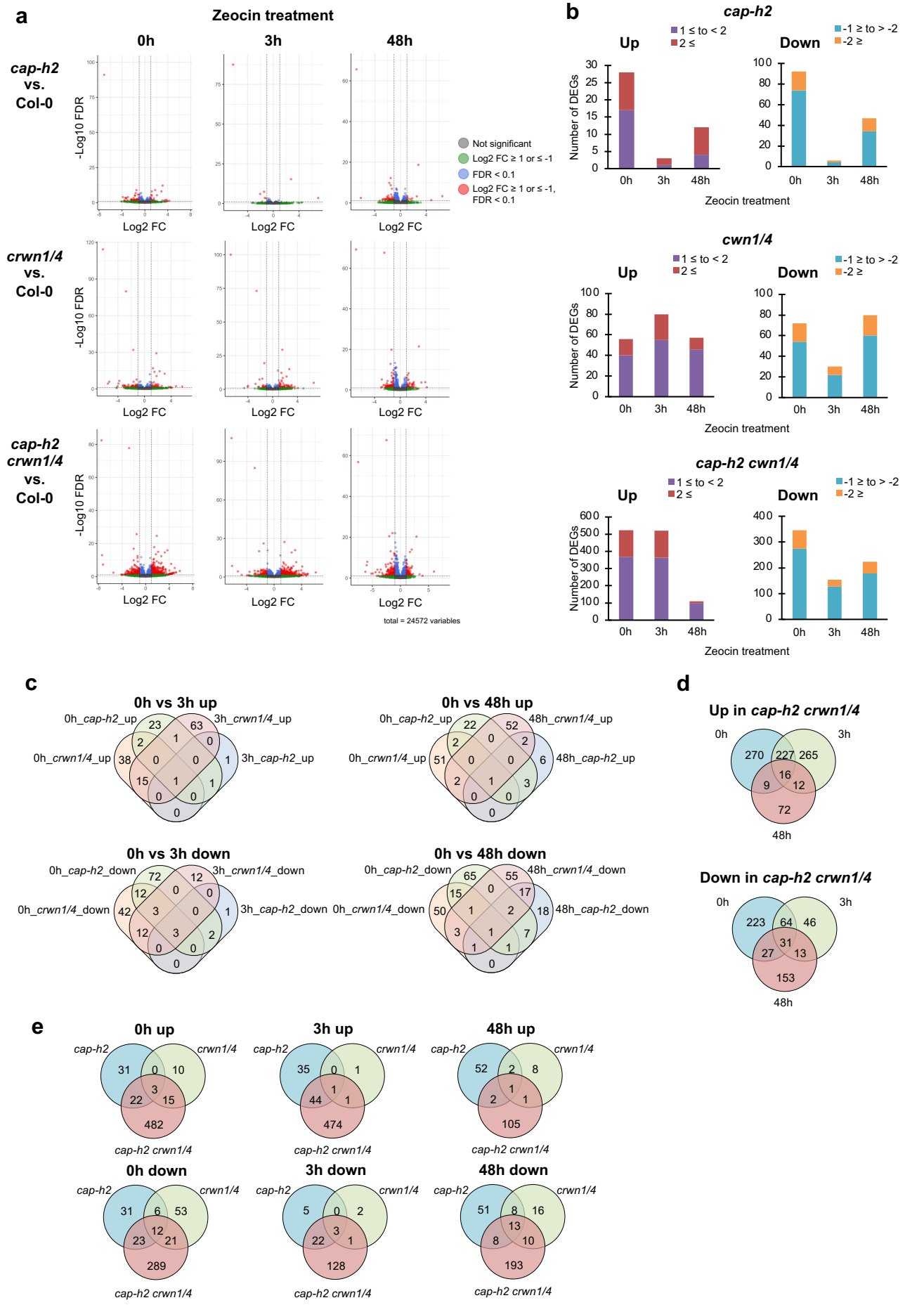
Supplementary Fig.10



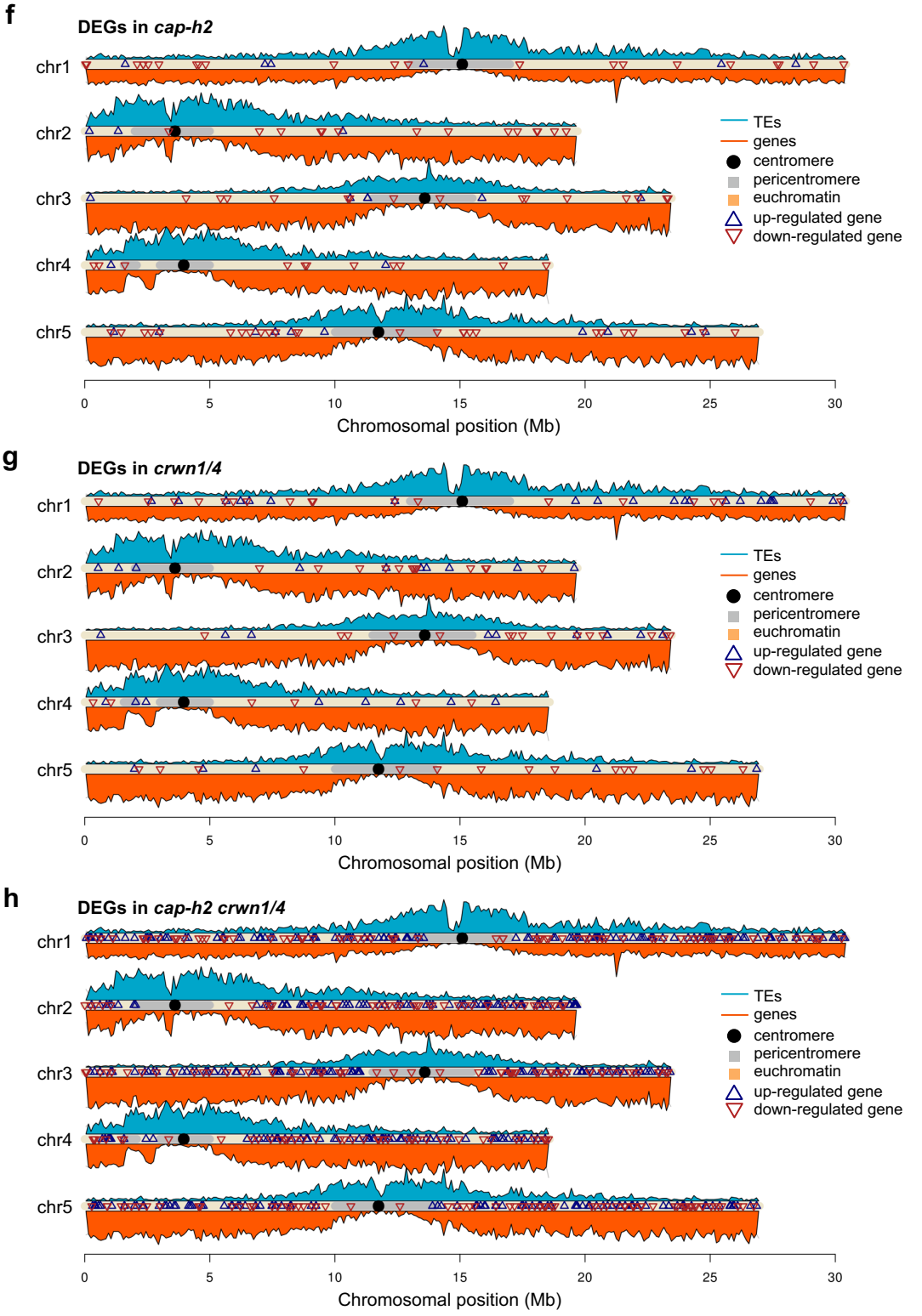
Supplementary Figure 10. Genes differentially expressed in the mutants showing abnormal centromere distribution, as assessed with microarray analysis

a, Volcano plots of the genes expressed in *cap-h2-2* (left panel) and *crwn1/4* (right panel). Total RNA was extracted from plants grown on the MGRL medium for 14 d and subjected to microarray analysis. **b**, Number of DEGs. Up- and down-regulated genes in the *cap-h2-2* and *crwn1/4* mutants compared to those in Col-0 are shown (Log2 FC ≥ 1 or ≤ -1, FDR < 0.1) (*n* = 3). **c**, Overlaps of DEGs between *cap-h2-2* and *crwn1/4*.

Supplementary Fig.11



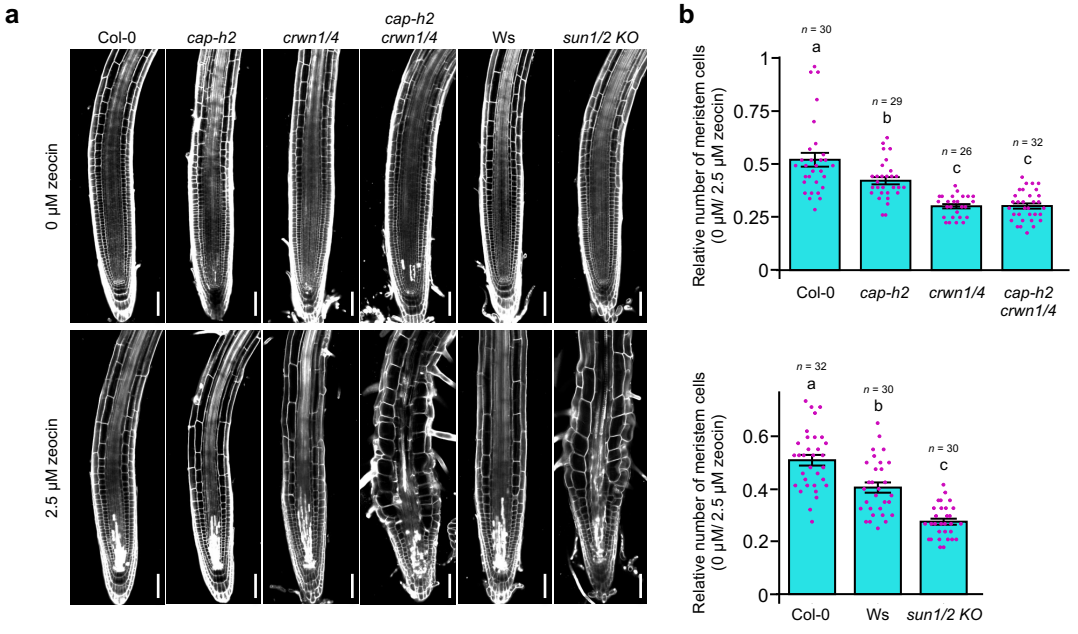
Supplementary Fig.11 continued



Supplementary Figure 11. Genes differentially expressed in response to DNA damage induction in the mutants showing abnormal centromere distribution, as assessed with RNA-seq analysis

a, Volcano plots of the genes expressed in *cap-h2-2 (*upper panel), *crwn1/4* (middle panel), and *cap-h2-2 crwn1/4* (lower panel). Total RNA was extracted from zeocin-treated plants and subjected to RNA-seq analysis. The details of the growth conditions are provided in the M&M section. **b**, Number of DEGs. Up- and down-regulated genes in the *cap-h2-2, crwn1/4*, and *cap-h2-2 crwn1/4* mutants compared to those in Col-0 are shown (Log2 FC ≥ 1 or ≤ -1, FDR < 0.1) (*n* = 3). **c**, Overlaps of DEGs between *cap-h2-2* and *crwn1/4* and between treatments. **d**, Overlaps of DEGs in *cap-h2-2 crwn1/4* between treatments. **e**, Overlaps of DEGs between *cap-h2-2, crwn1/4,* and *cap-h2-2 crwn1/4* for each zeocin treatment. **f–h**, Chromosomal locations of DEGs in *cap-h2-2* (**f**)*, crwn1/4* (**g**)*,* and *cap-h2-2 crwn1/4* (**h**) under control conditions.

Supplementary Fig.12



Supplementary Figure 12. Phenotypes of root morphology under zeocin treatment in the mutants showing abnormal centromere distribution

a, Effect of zeocin treatment on root tips. The roots were stained with propidium iodide. Fluorescence images of root tips treated with 0 or 2.5 μM zeocin for 2 d are shown. Bars = 200 μm. **b**, Effect of zeocin treatment on root meristem size. The number of meristematic cortex cells was counted using the images shown in (**a**). Values are ratios relative to values under normal conditions. The graph shows average ± s.e.m., $n \geq 26$ cortical rows, $P < 0.05$, one-way ANOVA and Tukey's HSD. Different letters indicate that the values are statistically different. Precise values of *n* are shown as discrete numbers.