Supplementary Materials for

Cell size controlled in plants using DNA content as an internal scale

Marco D'Ario, Rafael Tavares, Katharina Schiessl, Bénédicte Desvoyes, Crisanto Gutierrez,
Martin Howard, Robert Sablowski*

*Corresponding author. Email: robert.sablowski@jic.ac.uk

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Other Supplementary Material for this manuscript includes the following:
(available at science.sciencemag.org/content/372/6547/1176/suppl/DC1)

Datasets S1 to S3 (Excel)
MDAR Reproducibility Checklist (PDF)

Materials and Methods

Arabidopsis lines

Arabidopsis thaliana lines transformed with RBR1-GFP (20), KRP4-GFP (21), *CDT1-CFP* (16), *pUBQ10::acyl-YFP* (12), *NUP136-GFP* (31), *FBL17-GFP* (23) and *CLV3>>KRP4* (*pCLV3:LhG4 Op:GFP Op:KRP4*) (11) have been described. KRP4-mCherry was produced as explained for KRP4-GFP, except that the mCherry coding sequence (32) was used instead of GFP. The *fb17* mutant (GABI_170E02) (23) has been characterized; the loss-of-function *krp4-2* allele was isolated as a gene trap line (GT1143) in *L-er* background (33). For CRISPR-Cas9 mutagenesis, vectors were assembled using Golden Gate cloning as described (34, 35), using the guide RNA sequences corresponding to the primers listed in Supplementary Dataset S1 and the final vector pICSL4723 (36). To make plants with inducible loss of FBL17 function, the *FBL17*-amiRNA was designed and introduced into the *Arabidopsis* miR319a precursor by overlapping PCR (24) using the primers listed in Supplementary Dataset S1. The resulting precursor was assembled with LexA promoter (from pICSL12005; TSL SynBio; <http://synbio.tsl.ac.uk>), and OCS terminator (from pICH41432; addgene #50343; addgene.org) into a level 1 acceptor plasmid pICH47751 (addgene #48002). The vector pICSL80003 (TSL SynBio) harbouring the chimeric transactivator XVE (37) was assembled with the YAO promoter (from pICSL12039; TSL SynBio), and *rbcS-E9* terminator (from pICSL60004; addgene #117519) into the second level 1 acceptor plasmid pICH47742 (addgene #48001). These two level 1 vectors were assembled with the FAST-RED selectable marker (from pICSL11015; addgene #117499) to generate the estrogen-inducible construct in the final vector pICSL4723. *Arabidopsis* was transformed using the floral-dip method (38).

Genotyping

Leaf samples (approximately 1cm long) were ground in liquid nitrogen, resuspended in 200µl of DNA extraction Buffer (100 mM Tris-HCl, 320µM NaCl, 200 µM EDTA and 4% SDS) incubated for 1h at 50°C and centrifuged (12000 X g/5 min). 150 µl of the supernatant was mixed with 1 vol isopropanol; after 5 min at RT and 5 min at 12000 X g, the pellet was washed with 70% ethanol, dried and resuspended in 200 µl of water. 0.8 µl of this solution were mixed with 9.2µl of PCR mix (2.5mM dNTPs, 2.5mM genotyping primers listed in Supplementary Dataset S1, 1x Q5® Buffer and 0.02 U/µl of Q5® Polymerase, from New England Biolabs), and incubated in a thermocycler using the following setting: 98°C for 5min followed by 35 cycles of 98°C for 30sec, 58°C for 30sec and 72°C for 1min per kb and one cycle of 5min at 72°C.

Plant growth conditions

Arabidopsis plants were grown on JIC *Arabidopsis* Soil Mix (Levington F2 compost with Intercept and 4-mm grit at a 6:1 ratio) at 16°C under continuous light (100 µE). Plants were dissected at approximately 5-7 weeks after sowing, when the inflorescence stem was approximately 10-15 cm tall. For the *fb17* mutant, seeds were sterilized and plated on GM medium (0.1% glucose; 0.44% Murashige and Skoog Medium including Vitamins (Duchefa Biochemie), 0.9% agar; pH 5.7), homozygotes were selected based on their deformed cotyledons and short roots and confirmed by PCR genotyping as described above; shoot apices were imaged 8-10 weeks months after sowing.

Quantitative Reverse-Transcription PCR (qRT-PCR)

To measure miRNA inhibition of FBL17, 100-200 seeds per biological replicate were germinated and grown for seven days on filter paper with 10 µM β-estradiol (Sigma, E1024)

and 0.1% DMSO. Seedlings were ground in liquid N₂, total RNA was extracted with Tri-Reagent (Sigma T9424) and treated using Turbo DNase free kit (Invitrogen) as per the manufacturer's instructions. 1 µg of total RNA was reverse-transcribed using oligo(dT) (20-mer) and Super-Script IV reverse transcriptase (Invitrogen) according to the manufacturer's instructions. Relative expression analysis was performed with the SYBR GREENR JumpStart™ Taq ReadyMix™ (Sigma) and the primers listed in Supplementary Dataset S1, using a LightCycler 480 and associated software (Roche; version 1.5). Expression of *UBI10* (At4g05320) was used as the internal standard. Each biological replicate was measured as the average of three technical replicates.

Confocal imaging

For live imaging, apices were dissected and grown as done previously (11). In experiments using miRNA induction, apices were incubated in the same medium supplemented with 10 µM β-estradiol (Sigma, E1024) for 48 h before imaging. For time-course imaging, apices were transferred to medium with 50 µM cytokinin (*trans*-Zeatin, Sigma Aldrich, Z0876) as described (39) and allowed to recover at 20°C under continuous light for 24h, after which they were imaged every 2 hours for 24 or 48h, with resting periods in the same medium at 20°C under continuous light. Cell membranes were marked with *pUBQ10::acyl-YFP* (12) or stained with FM4-64 (N-(3-Triethylammoniumpropyl)-4-(6-(4-(Diethylamino) Phenyl) Hexatrienyl) Pyridinium Dibromide) (Thermo-Fisher, T3166) (11). For 4',6-diamidino-2-phenylindole dihydrochloride (DAPI, Sigma, D9542) imaging, dissected apices were incubated in 100 µg/mL of FM464-FX (Thermo-Fisher, F34653) for 20 min, infiltrated under vacuum with 3% (v/v) formaldehyde in phosphate-buffered saline (PBS) for 2 min on ice and 30 min at room temperature, washed 3 times in PBS, stained in 1 µg/mL of DAPI in PBS for 20 min, washed with PBS and water, then imaged as described (11). Confocal and Airyscan Fast Mode imaging (for time course imaging) were performed with a Zeiss LSM880 confocal microscope using a W Plan-Apochromat 40x/1.0 DIC M27 water dipping objective, with the following settings: for GFP, excitation with argon laser and band pass 420-480 nm, emission band pass filter 495-550; for YFP, excitation with argon laser and band pass filter 495-550 nm, emission long pass filter 570 nm; for mCherry and FM4-64, excitation with DPSS 561-10 laser and band pass filter 495-550 nm, emission long pass filter 570 nm; for CFP, excitation with argon laser and band pass filter 420-480 nm, emission long pass filter 525 nm. Laser power (2-12%) and gain were set according to individual samples to maximise signal without saturation but were kept the same for images that had to be compared.

Image analysis

Original and processed images used in each manuscript figure, along with metadata files and measurement tables, are listed on Supplementary Dataset S2 and can be downloaded using the DOIs provided. These images were processed (three-dimensional cell segmentation, cell tracking in time course experiments, measurement of cell volumes, fluorescent signal and cell positions in the meristem) using Python scripts and Fiji macros adapted from published scripts (11, 40, 41). Details of how to install and use the scripts, along with a description of each script and the annotated source code are available at <https://doi.org/10.6084/m9.figshare.14529372.v1>. The resulting raw data used in each figure, along with annotated Python scripts used to generate the graphs, are provided in Supplementary Dataset S3.

Numerical simulations

To simulate unsynchronized cell populations growing and dividing according to the assumptions in Figures 4A and S8A, the Python script `numerical_simulations.py` was used (annotated code available in <https://doi.org/10.6084/m9.figshare.14529390.v1>), which imports functions from Numerical Python (<http://www.numpy.org>), Scientific Python (<http://www.scipy.org>) and matplotlib (<http://matplotlib.org>). The script can be run by manually copying and pasting functions and command lines directly on a Python terminal, or by executing it from the terminal after editing parameter values and data paths in the script. Apart from parameter values, the script inputs included tables of experimental data for division symmetry, cellular growth rates and rates of KRP4 synthesis in G1; these tables can also be found in <https://doi.org/10.6084/m9.figshare.14529390.v1>. The main steps in the simulation were:

1. A small set of starting cells were created, with exponential growth rates, initial volume and rate of KRP4 synthesis extracted from the experimental data; to simulate the experimental variability of KRP4 levels during mitosis, values were picked at random from scaled experimental data; values for the mean amount of KRP4 on chromatin and density in the nucleoplasm at the start of the cell cycle were set as simulation parameters with values given in Fig. 4B.
2. The cells were allowed to grow for 1 hour iterations; during the first six hours, additional KRP4 was produced at experimentally measured rates (each pair of simulated sister cells was attributed synthesis rates from a random pair of experimental sister cells, with the larger and smaller simulated sisters receiving the rates of the larger and smaller experimental cell, respectively).
3. Growth iterations were repeated until the total KRP4 concentration dropped below a threshold, whose value was set to the median total KRP4 amount at birth divided by the median wild-type cell volume at the G1/S transition.
4. S and G2 proceeded for 20 hourly iterations, with a growth rate corresponding to the measured average rate in S/G2 (this resulted in a constant CV for volumes during S/G2, as observed experimentally).
5. At division, the ratio between daughter cell volumes was picked at random from experimental values and each daughter cell again received the amounts of KRP4 on chromatin and in the nucleoplasm set as in step 1, with added variability based on the experimental data.
6. The simulations ran for 800 iterations, with population cell volumes collected every 100 iterations; if the total population increased over 1000 cells, a random subset was deleted (equivalent to cells leaving the meristem).

Derivation of a mathematical model for KRP4 dynamics

To derive a mathematical description of the KRP4 dynamics in the nucleus, we consider only the nuclear population, as the imaging experiments indicated that the cytoplasmic fraction was negligible. Our model for KRP4 dynamics encodes the following reactions:

Here, K and C are free KRP4 and unbound DNA in the nucleoplasm, respectively, and KC represents the complex of KRP4 bound to the DNA. We denote by N_t the total number of KRP4 binding sites on the DNA. The total molecular numbers of KRP, unbound and bound DNA, respectively are represented by N_k , N_c and N_{kc} . We therefore have $N_c + N_{kc} = N_t$. The nuclear volume is given by V and the respective densities within the nucleus are given by ρ_k ,

ρ_c and ρ_{kc} . β is the rate constant for complex formation, while γ is the dissociation rate. ϕ is the KRP4 production rate per unit volume, with μ being the degradation rate, mediated at least in part of FBL17. Using the law of mass action, and properly accounting for cell volume, we can write equations for the association, dissociation, production and degradation reactions:

(2)

$$\begin{cases} \frac{dN_k}{dt} = -\beta \frac{N_k N_c}{V} + \gamma N_{kc} + \phi V - \mu N_k \\ \frac{dN_c}{dt} = -\beta \frac{N_k N_c}{V} + \gamma N_{kc} \\ \frac{dN_{kc}}{dt} = \beta \frac{N_k N_c}{V} - \gamma N_{kc} \end{cases}$$

As supported by experiments (Figure S5) [13, 14], we also take the nuclear volume to be proportional to the total cell volume.

We first examine these dynamics during the G2 phase of the cell cycle. We assume that the degradation timescale μ^{-1} of KRP4, as well as the KRP4 binding/unbinding timescale, are fast compared to the G2 cell cycle timescales. Note that it is not necessary for the unbinding timescale on its own to be fast: a scenario where KRP4 binds essentially irreversibly to the chromatin in G2 is also possible. The overall separation of timescales ensures that the system is in quasi-steady-state, in which case the derivatives in Eq. (2) can be set to zero. The nucleoplasmic KRP4 number is then simply $N_{k2} = \phi_2 V / \mu_2$, where the subscript ‘2’ denotes results and rates applicable to G2. Using this result to compute N_{kc2} from Eq. (2), we find:

(3)

$$N_{kc2} = N_{t2} \left(1 + \frac{\gamma_2 \mu_2}{\beta_2 \phi_2} \right)^{-1}.$$

Note that the molecular number N_{kc2} is constant as the cell grows through G2. This is expected as the nucleoplasmic background density of KRP4, N_{k2}/V , is constant.

We expect the above scenario to hold through G2, but changes in the rate constants can affect whether it is the nucleoplasmic fraction or DNA-bound fraction which dominates. We assume that during G2 the dominant fraction switches to DNA-bound, where N_{kc2} does not scale with cell size according to Eq. (3). It is straightforward to choose parameter values to satisfy these constraints (e.g. in Figure 4C, top panel).

We next carry these levels of KRP4 through into the G1 phase of the cell cycle. We first assume that in this phase KRP4 production and degradation stops ($\phi_1=0$, $\mu_1=0$, where the subscript ‘1’ denotes rates applicable to G1) and that the KRP4 association/disassociation rates again change, promoting more nucleoplasmic KRP4. Without production or degradation, the total amount of KRP4 is conserved and must satisfy

(4)

$$N_{k1} + N_{kc1} = \frac{\phi_2 V_0}{\mu_2} + \frac{1}{2} N_{kc2},$$

where N_{kc2} is from Eq. (3), using parameters appropriate for late G2 and where V_0 is the volume at the start of G1. The factor of one-half results from only half of the chromatin-bound KRP4 being inherited, with the other half ending up in the other daughter cell. We can then re-solve Eq. 2 without production and degradation, setting the derivatives on the left-hand side of Eq. (2) to zero, as appropriate for a rapid binding/unbinding timescale that is fast compared to the G1 cell cycle time. Rearranging these equations to express the volume in terms of the nucleoplasmic density ρ_{k1} as well as the other rate constants, we end up with:

(5)

$$V = \frac{N_{kc2}}{2\rho_{k1}} + \frac{\varphi_2 V_0}{\mu_2 \rho_{k1}} - \frac{\beta_1 N_{t1}}{\beta_1 \rho_{k1} + \gamma_1}.$$

Note that the case $\beta_1=0$ (or equivalently a large enough γ_1) is easily interpretable: in that case all the chromatin bound KRP4 will unbind, adding to the nucleoplasmic pool, with the sum diluting as the cell grows. For non-zero β_1 , some of the KRP4 remains on the chromatin, which when V is expressed in terms of ρ_{k1} , gives rise to the third term on the right-hand side of Eq. (5). However, from now on, for clarity of exposition, we will neglect this term in a low β_1 , high γ_1 limit.

We next assume that when the concentration of KRP4 in the nucleoplasm drops to a certain threshold value $\rho_{kl} = \rho_{ks}$ the G1/S transition is triggered, in an inhibitor dilution mechanism. For sizer behaviour, the size at which this occurs should be independent of the starting size V_0 . As can be seen from Eq. (5), this is true only when the second term can be neglected, requiring $\frac{1}{2} N_{kc2} \gg \frac{\varphi_2 V_0}{\mu_2}$, i.e. that there be much more KRP4 bound to the chromatin at the beginning of G1 than present in the nucleoplasm, requiring, for example, a sufficiently high β_2 . This result is again intuitive: only the chromatin-bound fraction at the beginning of G1 contains size information, as its molecular number is constant, meaning that when diluted out into the nucleoplasm, its density depends on cell size. For the nucleoplasmic fraction at the beginning of G1, its number already depends on the size V_0 at the beginning of G1. Hence, this component cannot readily store absolute size information.

After G1/S, the cell re-enters G2 with much diluted KRP4 levels. Production and degradation again kick in and rapidly return KRP4 levels to their quasi-steady-state values, as described in Eqs. (1) and (2). The separation of timescales in G2, with rapid binding/unbinding/degradation kinetics in both early and late G2, ensures that the system is reset ready for the next G1 phase.

One complication that we observed in our experiments is that KRP4 synthesis continues during the first part of G1. So far, our model has not incorporated this observation, which in principle could be detrimental to sizer behaviour. Interestingly, according to our experiments, the production of KRP4 in G1 does not scale with cell size (Figure S4H,I), contrary to the behaviour of most proteins. We do not seek to explain this interesting effect, but merely incorporate it phenomenologically into our model and examine whether sizer behaviour is compromised. We again study the case where the KRP4 association/disassociation rates change at the beginning of G1, promoting more nucleoplasmic KRP4, and assuming $\frac{1}{2} N_{kc2} \gg \frac{\varphi_2 V_0}{\mu_2}$, as before. We again assume that degradation is turned off at the beginning of G1, and that the additional KRP4 accumulates in the nucleoplasm at a fixed rate independent of size. Hence, if this initial phase lasts for a period τ , then an amount $\phi_1 \tau$ will accumulate, where ϕ_1 is the G1 production rate. Provided τ is constant, then a fixed absolute amount of KRP4 is added, adding to the fixed absolute amount of KRP4 which drops off the chromatin. The total is therefore also fixed and independent of size. It can therefore be subsequently diluted as previously, yielding information about absolute cell size that can be thresholded to trigger the G1/S transition. Hence, the early G1 production of KRP4 does not substantially interfere with the sizer mechanism, provided it is produced at a rate and for a duration that are independent of cell size.

Implementation of the mathematical model in computer simulations

To simulate the dynamics of KRP4 in G2 and proliferating cell populations according to the mathematical model shown in Figure 4C and S8, the Python script ODE_simulation.py

was used (annotated code available in <https://doi.org/10.6084/m9.figshare.14529390.v1>), which used functions from Numerical Python (<http://www.numpy.org>), Scientific Python (<http://www.scipy.org>) and matplotlib (<http://matplotlib.org>). The differential equations and rate constants used in the simulation corresponded to those shown in Figure S8. The parameter values used to simulate KRP4 dynamics and the output graphs are also shown in Figure 4C and below.

To simulate proliferating cells, the asymmetry of cell divisions and exponential growth rate were extracted from experimental data (available in Supplementary Dataset S5). In the initial mother cell, the initial volume, number of molecules of free KRP4, DNA-bound KRP4 and total DNA binding sites were set to $150 \mu\text{m}^3$, 100, 100 and 200, respectively. During G1, cells grew at a relative rate of 1.00025 min^{-1} , with the rates for KRP4 dynamics set to: $\beta_1 = 1.0 \mu\text{m}^3 \text{ min}^{-1}$, $\gamma_1 = 1.0 \text{ min}^{-1}$, $\phi_1 = 0 \mu\text{m}^{-3} \text{ min}^{-1}$, $\mu_1 = 0 \text{ min}^{-1}$. G1 ended when the KRP4 concentration dropped below $0.25 \text{ molecules}/\mu\text{m}^3$. In G2, the number of DNA binding sites was doubled, the rate of KRP4 release from chromatin was diminished ($\gamma_2 = 0.1 \text{ min}^{-1}$), KRP4 synthesis started ($\phi_2 = 0.1 \mu\text{m}^{-3} \text{ min}^{-1}$), balanced with degradation ($\mu_2 = 0.5 \text{ min}^{-1}$ for the wild type, $\mu_2 = 0.2 \text{ min}^{-1}$ to simulate *fbf1*). After a combined S/G2 period corresponding to 20 hours (the average time measured between G1/S and mitosis), a new cycle was started, where each daughter cell inherited half the number of DNA binding sites, half the number of KRP molecules bound to chromatin and a fraction of free KRP4 molecules proportional to volume at birth. After a total of 10 cycles, cell volumes were sampled at random times during the tenth cycle, to mimic an asynchronous cell population.

Supplementary Figures

Fig. S1.

Cell cycle and cell growth parameters inferred from live imaging.

(A, B) Boxplots and individual values (colored dots) of the number of mitoses (A) and the average relative growth (i.e., the volume of a cell divided by its volume an hour earlier) (B) measured at each time point in four meristems (266, 261, 236 and 165 cells measured, respectively); note that there may have been periodicity in cell division and growth rates, but no overall downward trend that would have suggested stress or photodamage due to repeated imaging.

(C) Boxplots of cell volumes from tracked cell lineages aligned at mitosis (542 cell lineages pooled from 4 meristems); the typical cell cycle length was considered to be the time taken to restore median cell volume (in the graph shown, from -34 to +34 hours relative to mitosis).

(D) Boxplots of CDT1a-CFP signal density in the same cell lineages shown in (A); G2 length was considered to be the interval between the large drop in CDT1a-GFP levels and the subsequent mitosis (20 h). The difference between the total cell cycle and G2 estimates from (A) and (B), respectively, was taken as the typical G1 length (48 h).

(E) Correlation between the median growth rate during the interval and the median starting volume at each time interval shown in (C), calculated as the slope of a linear regression of volume versus time; r and p are the correlation coefficient and p -value of a least-squares linear regression.

(F) Correlation between the relative growth in the subsequent 4 hours and the starting cell volume. The starting point was cell birth, when the lineage could be tracked at least four hours after mitosis, or otherwise, the earliest time when the lineage could be tracked; r and p are the correlation coefficient and p -value of a least-squares linear regression (576 cells pooled from 4 meristems).

(G) Difference between growth rates of larger and smaller sisters plotted against volume asymmetry (170 sister pairs pooled from 4 meristems). Growth rates were calculated as the exponential growth rate per hour during the first four hours after mitosis; green bars indicate the 95% confidence interval for the difference in growth rates for each asymmetry interval (calculated by bootstrapping, see dataset S3).

Fig. S2.

The concentration of RBR1-GFP increases with cell volume up to the average volume at the G1-S transition (200 μm^3 , Figure S1 A,B), whilst the concentration of KRP4-GFP decreases. (A, C) Confocal sections through a meristem expressing RBR1-GFP (A) or KRP4-GFP (C) (green signal), with cell outlines stained with FM4-64 (red signal); bars: 10 μm . (B,D) Segmented images corresponding to (A,C), with individual cells marked in random colors and regions where nuclear GFP signal was measured marked in white; bars: 10 μm . (E,F) Scatterplots of GFP signal density (sum of all fluorescent signal in the nucleus divided by nuclear volume) versus cell volume for RBR1-GFP (700 cells from 4 meristems, E) and KRP4-GFP (845 cells from 5 meristems, F).

Fig. S3.

Complementation of a *krp4* mutant with *KRP4-mCherry*

The loss of function *krp4-1* mutant does not have visible phenotypes in otherwise wild-type background, but partially rescues floral organ growth in the *jag-2* mutant, in which growth is inhibited by ectopic KRP4 (21). When introduced in *jag-2 krp4-1* plants, *pKRP4:KRP4-mCherry* restores the inhibition of organ growth, showing that the fusion protein provides *KRP4* function.

A-D: inflorescence apices of wild-type (A), *jag-2* (B), *jag-2 krp4-1* (C) and *jag-2 krp4-1* transformed with *pKRP4:KRP4-mCherry* (D); note the rescue of petal growth in (C), which was abolished in (D); bars: 1 mm.

Fig. S4.

Equal inheritance and asymmetric concentration of KRP4-GFP in sister cells, and dynamics of KRP4-mCherry accumulation in early G1.

(A,B) 3D segmented image of a representative meristem with pairs of sister cells marked in the same color; (A) sister cells verified by tracking mitoses over 48 hours; (B) sisters detected automatically based on cell geometry at the end of the time course; size bars: 10 μm .

(C) Quality control for sister cell detection; the Venn diagram shows the overlap between tracked sister cells (red) and automatically detected sisters (blue) (combined data from 4 biological replicates); the boxplots show sensitivity (percentage of tracked sisters correctly detected by geometry), accuracy (percentage of overlapping cell pairs in which automatic detection attributed the correct sister), and the number of automatically detected sisters for which mitoses were not seen during the time course.

(D,E) Optical section of meristem expressing KRP4-GFP (green signal) with cell outlines stained with FM4-64 (D) and corresponding segmented image (E), with sister cells assigned based on cell geometry (colored pairs of cells); blue and yellow arrows indicate symmetric and asymmetric sister pairs, respectively; bar: 10 μ m.

(F,G) Difference in KRP4-GFP signal density (F) and total signal (G) between larger and smaller sisters, plotted against volume asymmetry; blue bars indicate the 95% confidence interval (calculated by bootstrapping) for the difference for each asymmetry interval (243 sister pairs pooled from 5 meristems, assigned based on cell geometry, selected from L1-L2 layers, within 40 μ m of the meristem center and with a combined sisters volume below the population mean).

(H) Correlation between cell size at birth and the rate of KRP4-mCherry accumulation during the first 6 hours after birth (157 cells pooled from 3 meristems).

(I) Correlation between rate of KRP4-mCherry accumulation during the first 6 hours after mitosis in sister cells (101 cell lineages tracked in 3 meristems).

Fig. S5.

Nuclear volume is proportional to meristem cell volume.

(A,B) Orthogonal view of a stack of confocal images of a meristem expressing the nuclear membrane marker NUP136-GFP, with cell outlines marked with FM4-64 (A) and a corresponding stack with segmented nuclei (random colors) and cells (with cell boundaries shown in white); bars: 10 μm .

(C) Scatterplots of nuclear volume versus against volume in three NUP136-GFP meristems (blue, red and green points, with 139, 143 and 154 cells, respectively); the linear regression was fitted to all points combined.

Fig. S6.

Inhibition of *FBL17* expression using an inducible artificial miRNA

A: Schematic diagram of the system used to inhibit *FBL17* expression. The meristem-expressed *YAO* promoter was used to direct expression of the artificial transcriptional activator XVE (29). In the presence of estrogen, XVE can activate the LexA promoter, which directs expression of a modified precursor (24) that produces a miRNA targeted to the 3' region *FBL17*; cleavage by the miRNA is expected to cause degradation of the *FBL17* mRNA.

B: *FBL17* expression in the wild type and in miRNA-expressing lines, measured by qRT-PCR in seedlings germinated in the presence of estrogen, using *UBI10* mRNA as the internal reference and normalized to the average value for the wild-type; the position of *FBL17* primers is shown by purple arrowheads in (A); bars and error bars show the average and standard deviation of three biological replicates for each line. Note that independent lines showed different levels of *FBL17* inhibition; among lines segregating the miRNA construct as a single locus, line 4 was selected for further experiments because it showed the strongest effect (70% reduction in average expression compared to wt, $p = 1.06 \times 10^{-2}$, Welch's *t*-test).

Fig. S7.

Mitotic chromosomes are associated with comparable levels of KRP4-GFP in control and in meristems with miRNA-inhibited *FBL17*. Note that given the observed increase in both KRP4 levels and cell sizes, the expected change in the concentration in cytoplasmic KRP4 would be difficult to detect against the background signal. For example, if the total KRP4 increased by an amount equal to that present in wild type cells at the end of G2, the combined effect of diluting the excess KRP4 in a cell twice as large, in a cytoplasm five times larger than the nucleus, would produce an increase in cytoplasmic concentration corresponding to 10% of the already low nuclear concentration seen in wild-type late G2 (Figure 2B,E). (A-D) Confocal sections of meristems expressing KRP4-GFP from a wild-type plant (A) and plant with miRNA-inhibited *FBL17* (C), counterstained with FM4-64, next to corresponding sections after 3D segmentation (B,D); white areas in (B,D) correspond to regions where GFP signal was measured in each cell; arrows show metaphase plates; bars: 10 μm . (E, F) Boxplots of volumes (E) and GFP signal (F) corresponding to whole cells, mitotic chromosomes and cytoplasm in cells undergoing mitosis (like those indicated by arrows shown in A-D) in the wild type (blue, 7 cells from 5 meristems) and after miRNA inhibition of *FBL17* (orange, 11 cells from 5 meristems).

Fig. S8.

Overview of numerical simulations and mathematical model.

A) Main steps of the numerical simulations; the equation below (re-arranged from the equation used to set the end of G1 in the simulations) summarizes how cell volume in S-phase (V_s) depends on KRP inherited on chromatin (N_{kc2}) and in the nucleoplasm (ρ_F).

B) Mathematical model: the diagram shows part of a nucleus with symbols as in (A); arrows indicate KRP production, degradation, binding and unbinding to chromatin with rate constants ϕ , μ , β and γ , respectively. The differential equations describe the dynamics of KRP4, with variables N, V, t and indices $k, c, kc, 0, 1, S, 2$ as detailed. The derived equation below (see Supplementary Methods) expresses how size at G1/S depends on KRP4 inherited on chromatin (magenta) and in the nucleoplasm (green) and on chromatin binding during G1 (black); the terms in magenta and green are equivalent to those in (A).

Fig. S9.

Effect of loss and gain of *KRP* function on cell volumes.

(A) Diagram of genetic lesions in *KRP3* and *KRP5* alleles generated by CRISPR-Cas9 mutagenesis. The arrows are transcripts, with black boxes and lines as exons and introns, respectively. The positions of nucleotides deleted in each allele are shown above the transcripts, and the corresponding changes in protein sequence are shown below.

(B) Boxplots showing cell size distributions (L1 and L2 layer cells within 40 μm of the meristem center) in wild-type, *krp4-2*, *krp4-2 krp3* and *CLV3>>KRP4* meristems; p-values are from pairwise comparisons to the wild type using the two-tailed Mann-Whitney test.

(C-F) Distributions of the relative deviation from mean volume and coefficient of variability (CV) of volumes for the sets of cells used in (B).

Fig. S10.

Evidence that the large meristem cells of the *fbl17* mutant are not polyploid.

(A-C) Optical sections of meristems stained with DAPI and counter-stained with FM4-64-FX, showing uniform DAPI intensity in the wild type (A), diluted DAPI signal in large cells of *fbl17* (arrows in B) and uniform DAPI signal in control polyploid cells (colchicine-treated wild type, C); note the large dividing cell in *fbl17* (yellow asterisk in B); size bar: 10 μm.

(D, E) Plots of nuclear volume against DAPI fluorescence in wild-type (D, 473 cells from 5 meristems) and *fbl17* (E, 273 cells from 9 meristems); images were taken with the same settings, nuclei were selected from epidermal cells within 30 μm of the meristem center and checked manually for correct segmentation; note the wild-type pattern consistent with dilution by growth in G1, duplication in S phase and dilution again in G2, whilst the mutant showed a wider spread of nuclear sizes and generally low DNA concentration in large nuclei.

Captions for Datasets S1 to S3 (Separate Excel Files)

Dataset S1

Sequences of oligonucleotides used for CRISPR-Cas9 mutagenesis, construction of the inducible miRNA construct, PCR genotyping and qRT-PCR.

Dataset S2

Imaging data used for each manuscript figure.

Dataset S3

Raw data used in manuscript figures and scripts used to generate the figures.

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