Supplementary information to: Macromolecular crowding limits growth under pressure

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I. STRAINS USED IN THIS STUDY

Name	background	Genotype
40nm-GEMs HTB2-mCherry	BY4741	HTB2::HTB2-mCherry-URA3; PINO4::Pfv-GS-Sapphire-LEU2
cytosolic GFP	W303	ura3::pRS306-pHIS3-2xGFP
nuclear envelop mCherry	W303	nup57::NUP57-mCherry-kanMX
20nm-GEMs	W303	ura3::PHIS3-AqLumSynth-Sapphire
mRNA particles	W303	GFA1/gfa1::GFA1-24PP7; PP7-CP-3xYFP::HIS3
DNA locus	W303	leu2::TETR-GFP:LEU2 ura3::TETOarray:URA3
P_{ADH2}-*mCherry*	BY4741	ura3Δ0::pADH2-mCherry-URA3; pINO4::Pfv-GS-Sapphire-LEU2
P_{ADH2}-*mCherry hog1*Δ	BY4742	hog1d::G418; pADH2-mCherry(URA)
40nm-GEMs *hog1*Δ	BY4742	hog1d::G418; leu2::PINO4-PfV-GS-Sapphire-LEU2; pADH2-mCherry(URA)

II. MATHEMATICAL MODELING

A. GIP compresses the nucleus by increasing intracellular colloid osmotic pressure.

We assumed that the nucleus was a passive osmometer in osmotic equilibrium with the cytoplasm. $\Pi_i^c = \alpha\Pi_i$ is the cytosolic colloid osmotic pressure (due to macromolecules that cannot freely diffuse through the nuclear pore), which is hypothesized to be proportional to the intracellular total osmotic pressure Π_i with a factor α. We denote Π_n the nuclear colloid osmotic pressure. Osmotic equilibrium enforces: $\Pi_i^c = \Pi_n$. When growth-induced pressure P builds up, the cytoplasmic total osmotic pressure increases from the nominal value Π_0, such that $\Pi_i = \Pi_0 + P$. Similarly, we assume that the cytosolic colloidal pressure increases to $P^c = \alpha P$, and $\Pi_0^c = \alpha\Pi_0$. This corresponds to an increase in osmolyte concentration from N_0 to N_P, while keeping the cell volume constant at v_c. We hypothesized that the change in cytosolic pressure compresses the nuclear volume, such that it decreases from v_n to $v_n + \delta v_n$. We also hypothesized that, during the buildup of GIP, there was no large increase in nuclear colloid osmotic pressure, osmotically active species remaining constant at N_n^0.

From van't Hoff's equation, under confined growth, osmotic equilibrium leads to,:

$$\frac{N_P}{v_c} = \frac{N_n^0}{v_n + \delta v_n} \tag{1}$$

Re-writing $N_n^0 = (v_n/v_c)\, N_0$ from the equilibrium without GIP, we determine the nuclear volume dependence with P to be:

$$\frac{\delta v_n}{v_n} = -\frac{P^c/\Pi_0^c}{1 + P^c/\Pi_0^c} = -\frac{P/\Pi_0}{1 + P/\Pi_0} \tag{2}$$

We used Eq. 2 to fit the experimental data of Fig. 1c, and extracted $\Pi_0 = 0.95 \pm 0.05$ MPa.

B. Change in cell volume after GIP relaxation.

We assumed, as above, that the intracellular osmotic pressure, Π_i, rose with GIP: $\Pi_i = \Pi_0 + P$, where P is growth-induced pressure. From our data, we found that cell volume, v_c^0, did not significantly change under confined growth. Growth under pressure is therefore accompanied with an increase in osmolyte concentration, from N_0 to N_P under a pressure of P. The van't Hoff equation gives:

$$(\Pi_0 + P)\, v_c^0 = N_P RT \tag{3}$$

R is the gas constant and T is the temperature. After pressure relaxation, the volume increased by a value δv_c, equilibrating intracellular osmolarity back to the control value Π_0. This can be written to $(\Pi_0 + P)\, v_c^0 = \Pi_0 \left(v_c^0 + \delta v_c\right)$, leading to:

$$\frac{\delta v_c}{v_c^0} = \frac{P}{\Pi_0} \tag{4}$$

Eq. 4 was used to predict the volume change after pressure relaxation, performed by devices similar to those previously published in [1]. These predictions are displayed in Fig. 1f.

C. Measuring growth rate from the experimental data.

We defined the growth rate k_g of the cell population, in terms of total cell volume V_c, as:

$$\frac{\partial_t V_c}{V_c} = k_g = \frac{\partial_t N}{N} \tag{5}$$

where N is the cell number. Indeed, since the single cell volume v_c^0 remained roughly constant (Fig. 1b), $V_c = Nv_c^0$. Writing that $N = \rho V$, with ρ being the cell density and V the volume of the PDMS chamber, one gets:

$$k_g = \frac{\partial_t N}{N} = \frac{\partial_t \rho}{\rho} + \frac{\partial_t V}{V} = \frac{\partial_t \rho}{\rho} + \frac{\partial_P V}{V}\partial_t P \tag{6}$$

The dependence of k_g on pressure has two contributions: cell density, and total volume. We measured both contributions independently:

- Contribution of cell density: we measured cell density in the chamber by counting cell nuclei using an *HTB2-mCherry* fluorescent histone marker.
- Contribution of cell volume: we measured the deformation of the chamber using bright-field imaging [1, 4], to infer growth-induced pressure.

Fig. S**??** plots the two respective contributions from Eq. 6. Note that cell density increases quickly in the chamber, suggesting that cells are more deformable than the PDMS chamber walls.

D. Protein expression from P_{ADH2}-*mCherry*.

We developed a simple model of protein production to extract key parameters from our fluorescence reporter assay. We hypothesized that, upon switch to carbon starvation, following a time delay t_d, transcripts m were produced with a rate k_m:

$$\partial_t m = k_m \tag{7}$$

and proteins p were produced from these transcripts with a net rate $k_p m$:

$$\partial_t p = k_p m \tag{8}$$

After an induction time t_d before which no mRNAs or proteins are produced ($m(t_d) = 0$ and $p(t_d) = 0$), Eq. 7 can be solved, giving $m(t) = k_m(t - t_d)$ predicting a quadratic increase of protein concentration with time, solving Eq. 8, as $p(t) = 1/2k_m k_p(t - t_d)^2$. Note that both k_m and k_p are effective net rates that incorporate multiple processes including transcript and protein degradation, export of the transcript from the nucleus, and fluorescent protein folding/maturation. It is impossible, from this simple fit, to extract more detailed information on which of these precise parameters is limiting for protein expression. We define instead an overall effective rate of protein production k_{exp}, such that $p(t) = k_{\text{exp}}(t - t_d)^2$.

Fluorescence intensity is proportional to the concentration of proteins $I(t) = \alpha p(t)$. The proportionality coefficient α cannot be readily predicted, and will depend on illumination conditions (intensity of the source, filters, exposure time, efficiency of the camera) and the fluorophore itself, therefore absolute protein concentration is not determined. However, the fitting of the data allows the extraction of a relative αk_{exp} for a given GIP condition. We kept the illumination conditions constant, and hypothesized that α does not depend on GIP. Renormalizing the rate for different values of pressure by the rate of the control thus allows for an estimation of the change in protein expression under GIP. Note α for mCherry does not vary significantly with crowding, in contrast to YFP *in vitro*[5].

Note on mRNA degradation: In this model we neglected mRNA degradation. Classically, the degradation rate of mRNA can be written $\partial_t m = -k_d m$. Incorporating it in Eq. 7 and solving mathematically $p(t)$, we find that, at long timescales, protein concentration should increase linearly in time following the relationship: $p(t) \sim k_m k_p / k_d t$. This linear regime is not observed experimentally, probably because we do not reach the linear regime in this experiment. At shorter timescales, including mRNA degradation, protein concentration reduces to $p(t) = 1/2k_m k_p t^2 + o(t^2)$, which is the mathematical function that we used to fit our data. The temporal range of the experiment does not appear to be large enough to extract a degradation rate and assess if this parameter changes under GIP.

E. Protein expression from P_{HIS3}-*GFP*.

We performed additional experiments on housekeeping genes to complement our results on protein synthesis with P_{ADH2}-*mCherry*. We investigated the dynamic change in fluorescence intensity of a GFP expressed from the constitutively active promoter P_{HIS3}-*GFP*. The increase in fluorescence over time can be analyzed using the following minimal assumptions:

1. Housekeeping protein production (e.g. His3p) follows the exponential cell volume increase such that protein concentration, N, increases exponentially in time[6]: $\partial_t N = k_{\text{prod}} N$.

2. Fluorescence intensity, I, is proportional to protein concentration, c.

Because $\partial_t I/I = \partial_t c/c = \partial_t N/N - \partial_t v/v = \partial_t N/N - k_{\text{growth}}$ where v is the cell volume and k_{growth} is the cellular growth rate, we can write that the derivative of the integrated fluorescence intensity as $\partial_t I/I = k_{\text{prod}} - k_{\text{growth}}$. Note that, any potential dependence of the fluorescence properties of GFP on crowding during the instantaneous quantification of protein expression from the HIS3 promoter, which occurs with almost no change in pressure or crowding, is thus normalized in this assay at almost constant pressure.

We measured the fluorescence intensity of the GFP over time and computed k_{prod} as a function of GIP according to the above equation. We superimposed this result to the P_{ADH2}-*mCherry* experiment (Fig. S**??**), and observed a very good agreement between these data.

F. Exponential dependence of nanoparticle diffusion with growth-induced pressure.

The Doolittle equation has been used previously to successfully relate the diffusion coefficient of nanoparticles to crowding inside the cell[7]. Denoting D_{w} as the diffusion coefficient of the nanoparticle in water, the diffusion coefficient D of a tracer nanoparticle can be expressed as[8]:

$$D = D_{\text{w}} \exp\left(-\xi \frac{v_{\text{crowder}}}{v_{\text{free}}}\right) \tag{9}$$

where v_{crowder} is the volume occupied by macromolecules and v_{free} is the free volume. The prefactor ξ relates to interactions between the tracer particle and the environment. We denote v^0_{crowder} and v^0_{free} respectively as the volume occupied by crowders and the free volume in the control (uncompressed) condition, for which $D = D_0$. We can re-write D as:

$$D = D_0 \exp\left(-\xi \left(\frac{v_{\text{crowder}}}{v_{\text{free}}} - \frac{v^0_{\text{crowder}}}{v^0_{\text{free}}}\right)\right) \tag{10}$$

We assume that cells are mainly crowded at the mesoscale by ribosomes of volume v_r, at a cytosolic concentration c_r that was previously estimated as $\sim$ 14,000 ribosomes / μm^3 [7]. In the cytosol, v_{free} refers to the cytosolic volume, such that $v_{\text{crowder}}/v_{\text{free}} = c_r v_r$. Note that this rewrites the number of ribosomes, contained in v_{crowder}, in terms of its concentration. Denoting c^0_r the control crowder concentration, we get:

$$D = D_0 \exp\left(-\xi c^0_r v_r \left(\frac{c_r}{c^0_r} - 1\right)\right) \tag{11}$$

Based on our experiments, we assume that the concentration of osmolytes, c_o, is proportional to the concentration of crowders, such that $c_r/c^0_r = c_o/c^0_o$. Using the van't Hoff equation to relate the concentration of osmolytes to the osmotic pressure, and denoting Π_i the intracellular osmotic pressure, we can re-write the diffusion of 40nm-GEMs as:

$$D = D_0 \exp\left(-\xi c^0_r v_r \frac{\Pi_i - \Pi_0}{\Pi_0}\right) = D_0 \exp\left(-\frac{\xi c^0_r v_r}{\Pi_0} P\right) = D_0 \exp\left(-P/P_c\right) \tag{12}$$

where P is the growth-induced pressure, defined as the intracellular osmotic pressure accumulated above the control value $P = \Pi_i - \Pi_0$, and $P_c = \Pi_0/(\xi c^0_r v_r)$ is a characteristic pressure associated with the exponential decay of the tracer particle. The inverse relationship between P_c and particle size s displayed in Fig. 1e suggests that $\xi \propto s$: the larger the nanoparticle, the larger the steric interactions, therefore the greater the effect of crowding. In principle, ξ can also incorporate other types of interactions, such as electrostatic interactions.

G. Calibration of the motion of 40nm-GEMs.

We used osmotic perturbations to calibrate the 3 parameters that define P_c for 40nm-GEMs: ξ_{40}, $c_r^0 v_r$ and Π_0. We determined the intracellular osmotic pressure as $\Pi_0 \sim 0.95$ MPa from the compression of the nucleus (Fig. 1c). Approximating the ribosome as a sphere with a radius of 15 nm, $c_r^0 v_r \sim 0.2$ [7]. ξ_{40} can be determined by instantaneously changing cell volume using osmotic compression. The volume of water in cells can be written $v_\mathrm{w} = v_\mathrm{c} - v_\mathrm{no}$, where v_c is the cell volume and v_no is the non-osmotic volume of the cell, which corresponds to the volume occupied by the dry mass of the cell.

Keeping the number of crowders constant, Eq. 11 can be re-written:

$$D_{40} = D_0 \exp\left(-\xi_{40} c_r^0 v_r \left(\frac{v_w^0}{v_w} - 1\right)\right) = D_0 \exp\left(-\xi_{40} c_r^0 v_r \left(\frac{1 - v_\mathrm{c}/v_\mathrm{c}^0}{v_\mathrm{c}/v_\mathrm{c}^0 - v_\mathrm{no}/v_\mathrm{c}^0}\right)\right) \tag{13}$$

$v_\mathrm{no}/v_\mathrm{c}^0$ corresponds to the minimum cell volume after an osmotic compression, which we found to be about 0.4, in good agreement with previous data [7, 9]. We fitted the experimental data with Eq. 13 to extract $\xi_{40} = 7.4 \pm 2.5$ (Fig. S**??**). This allowed the estimation of the exponential dependence of diffusion with pressure (the characteristic pressure) to be $P_c = 0.64 \pm 0.2$ MPa. Using this value led to an excellent prediction of the effective diffusion of 40nm-GEMs as a function of GIP (Fig 1d.)

H. Modeling confined growth.

We developed a model to physically explain the phenomenology of confined cell growth. Here, we expand upon the basic ideas of cell growth, our hypothesis on what happens when cells are confined, and how we calibrated all the parameters of the model, leading to remarkable predictive power.

a. Fundamental concepts of the confined cell growth model. It has been shown that cell density remains remarkably constant as cells grow[10]. Thus, undertaking this as a starting point, in the absence of confinement, the rates of cell volume expansion and macromolecule biogenesis should be equal to maintain this constant macromolecular crowding. Cell volume expansion requires production of intracellular osmolytes, while macromolecule production requires many metabolic processes, but is limited by the production of macromolecules, including ribosomes, and subsequently proteins[6]. Indeed, we experimentally determined that growth rate was proportional to protein production rate. Thus, osmolyte and macromolecule production must be proportional to enable balanced growth. It remains unknown how exactly this proportionality is achieved, and this fundamental question is not addressed here. However, our data support the hypothesis that confined growth does not alter this proportionality.

According to this central hypothesis, cells will accumulate osmolytes and macromolecules at a proportional rate. When volume expansion is inhibited by confinement, the accumulation of macromolecules leads to an increase in crowding, while the accumulation of osmolytes results in an increase in intracellular osmotic pressure. We postulated the following sequence to describe growth in a step-by-step manner:

1. Cells produce macromolecules and osmolytes at a fixed cell volume, increasing intracellular osmotic pressure above the unconfined control value Π_0, $\Pi_i > \Pi_0$. The production of macromolecules is exponential, for example because ribosomes are rate limiting for the production of ribosomal proteins[6]. Per our central coupling hypothesis, this means that osmolyte production is also exponential. We denote this rate k_prod.

2. When the pressure difference between intracellular and extracellular osmolarity, $\Pi_i - \Pi_e$, exceeds a threshold value, the cell wall expands enough to allow the incorporation of new cell wall components[11, 12], leading to a cell volume increase of δv. The actual value of δv is not a critical parameter: it sets the time interval δt required to reach the threshold concentration of osmolytes, such that $\delta v/v = k_\mathrm{prod}\delta t$. $\delta v/\delta t * 1/v$ sets the growth rate of the cell.

3. The resultant increase in volume partly dilutes osmolytes (and macromolecules) below the threshold value required for further cell wall expansion. Osmolytes then again accumulate at fixed volume, and the sequence is repeated.

This sequential model of cell growth naturally leads to exponential growth at a constant level of macromolecular crowding, and the intracellular osmotic pressure is continually maintained around the threshold value for cell wall expansion. **We posit that the critical pressure for cell expansion is related to the cellular turgor pressure** P_t, which corresponds to the hydrostatic pressure that balances the elasticity of the cell wall. Within this framework,

if the elasticity of the cell wall were to increase, the critical pressure would also increase and so more osmolytes would be required to reach this new threshold. Since osmolyte and macromolecule production are coupled, macromolecular crowding would also necessarily increase. We hypothesize that this is what happens when cells are confined.

b. Modeling the elasticity of the confining environment. When the chamber becomes filled such that all the cells are in contact, cell expansion must overcome the additional elastic resistance of neighboring cells and the walls of the PDMS chamber. These elasticities can be modeled as two springs acting in parallel. Denoting E_c as the cell elasticity, and E_{PDMS} as the elasticity of the PDMS chamber, **the cell will be pushing against a material of an effective elasticity equivalent to** $E_{\mathrm{eq}} = E_c\, E_{\mathrm{PDMS}}/(E_c + E_{\mathrm{PDMS}})$.

PDMS operates in a linear elastic regime for small deformations, such that E_{PDMS} is a constant. This is not the case for the elasticity of the cells E_c. We must account for two effects: First, when all cells are tightly packed, the elasticity of each cell is related to its internal pressure[13], i.e. the osmotic pressure difference $\Pi_i - \Pi_e$. Thus, as GIP increases, the elastic resistance imposed by neighboring cells will also increase, and the cells will progressively become stiffer. Second, at lower cell densities (prior to becoming completely packed) some of the mechanical pressure will deform cells, until the density reaches a maximum. Taking these two factors together, to a first approximation, we hypothesize that $E_c = (\Pi_i - \Pi_e)\,(\rho(P) - \rho_c)\,/\rho_m$, where $\rho(P)$ is the pressure-dependence of cell density, ρ_c is the cell density at confluence, and ρ_m is the maximum cell density.

To evaluate how cell density depends on external pressure, we consider a volume V_0 filled with cells, occupying a volume V_c, and culture medium, occupying a volume V_m, such that $V_0 = V_c + V_m$. By definition, $\rho = 1/V_0$. We found that under confinement, cell volume is not strongly affected by growth-induced pressure. Therefore, if we compress a box of volume V_0 (leading to an increase in cell density), only V_m will decrease. The culture medium volume decreases because it is displaced by an increasing number of cells that are deforming at fixed volume. We hypothesize, as above, that cell deformability is $\Pi_i - \Pi_e$, such that:

$$\frac{\partial_P V_m}{V_m} = -\frac{1}{\Pi_i - \Pi_e} \tag{14}$$

where $P = \Pi_i - \Pi_0$ is the growth-induced pressure. We take the derivative of $\rho(P)$ and get the following differential equation describing the change in cell density as a function of P:

$$\partial_P \rho/\rho_m = \frac{1}{\Pi_i - \Pi_e}\rho/\rho_m\,(1 - \rho/\rho_m) \tag{15}$$

Eq. 15 has a sigmoidal solution:

$$\frac{\rho}{\rho_m} = \frac{1}{1 + \exp\left(-P/\left(\Pi_i - \Pi_e\right)\right)} + \left(\frac{\rho_c}{\rho_m} - \frac{1}{2}\right) \tag{16}$$

where ρ_c is the cell density when the cells are at confluence. **We posit that, after cell confluence is reached, the threshold pressure needed to further expand the cell wall increases as as a function of the compressive stress imposed by the surroundings**:

- Before confluence, this threshold pressure is the nominal turgor pressure P_t
- After confluence, the threshold pressure is increased by the additional stress required to deform the elastic surroundings, $E_{\mathrm{eq}}\delta V/V_{\mathrm{confluence}}$

where $V_{\mathrm{confluence}}$ is the total cell volume when confluence is reached. Note that this is conceptually similar to increasing cell wall elasticity. The extra compressive stress $P = E_{\mathrm{eq}}\delta V/V_{\mathrm{confluence}}$, that needs to be overcome to continue to expand in volume, is the growth-induced pressure: the larger the volume expansion δV is, the larger P becomes.

c. Feedback: inhibition of protein production (k_{prod}) by macromolecular crowding. As cell volume expansion becomes inhibited due to confinement, both osmolarity and macromolecular crowding increase. Our data indicate that protein production is diffusion-limited and decreases as macromolecular crowding increases (Fig 2d). We incorporated this feedback into our model, with the form:

$$k_{\mathrm{prod}} = k_0 \exp\left(-\xi c_r v_r\right) \tag{17}$$

where the protein production rate decreases, similarly to Eq. 11, as macromolecular crowding increases c_r (v_r corresponds to the volume occupied by a single crowder, see above).

d. Calibration of model parameters. This model has a large number of parameters: k_0, ξ and $c_r^0 v_r$ that define the production rate and its dependence on macromolecular crowding, ρ_c/ρ_m related to the definition of the cell elasticity, E_{PDMS}, the surrounding elasticity, Π_e and Π_0, the external and internal control osmotic pressure, and P_t the turgor pressure.

In the following, we describe the experimental calibration of all of these parameters (except P_t, which is described in the next section):

- Growth parameters: $c_r^0 v_r$ has already been calibrated, see above. Moreover, since k_{prod} is proportional to the growth rate, we calibrated k_0 such that $k_{\mathrm{prod}} = k_0 \exp(-\xi c_r v_r) = 0.3\ \mathrm{h}^{-1}$, which corresponds to the unconfined growth rate. Concerning ξ, we used the fact that P_c was inversely proportional to the size of the diffusing probe s, leading to $\xi \propto s$. Using the ξ_{40} value measured for the diffusion of 40nm-GEMs, and the observed limiting size of 92 nm (Fig. 3b), this gives $\xi = 92\,\xi_{40}/40 = 17 \pm 5.5$. This value led to $P_c \sim 0.28$ MPa, in excellent agreement with experimental data (Fig. 4b).

- ρ_c/ρ_m was calculated from cell number data (Fig. 4d), where we found that confluence was reached at about 40% of final cell number, leading to $\rho_c/\rho_m \sim 0.4$, consistent with previous data (see Fig. S2C from [4]).

- $E_{\mathrm{PDMS}} = 0.8 \pm 0.1$ MPa was calibrated by measuring the deformation of the chamber with a defined hydrostatic pressure.

- We measured the osmolarity of SCD medium at 30^{o}C, Π_e, using an osmometer, and found $\Pi_e = 0.63 \pm 0.05$ MPa.

- As described previously, we fitted the dependence of nuclear volume on GIP to estimate the nominal intracellular osmotic pressure, $\Pi_0 = 0.95 \pm 0.05$ MPa.

- Calibration of the turgor pressure was a complex process, requiring a combination of electron microscopy, AFM, and laser ablation (details below). After careful evaluation, we found $P_t = 0.05 \pm 0.02$ MPa.

Important note on $\Delta\Pi$ *vs.* P_t:
We do not find, as often assumed, that the osmotic pressure difference is equal to the turgor pressure, but instead that $\Delta\Pi = \Pi_0 - \Pi_e > P_t$. This result is not inconsistent, and actually points to common misconceptions made in the evaluation of these parameters in the literature. In fact, during rapid growth, the continual increase in cell volume leads to a pressure loss. This pressure reduction effect is known as the suction pressure. Thus, the pressure change is written: $\Delta\Pi = P_t + P_s$, where P_s is the suction pressure, corresponding to the pressure loss through water influx into the cell as cell volume expands. In the limit where cell volume cannot increase, the osmotic pressure difference is exactly the same as the turgor pressure. However, if cell volume is increasing, $\Delta\Pi > P_t$. From our measurements, we estimate the suction pressure to be $P_s \sim 0.27$ MPa. Does this value make sense? We were unable to find values in the literature, but we can estimate a reasonable value from known properties of *S. cerevisiae*. We denote R_h as the hydraulic resistance of the cell wall, and get $P_s \sim R_h J$, where J corresponds to the flow of water, which is approximately the same as the cell volume expansion rate. Hydraulic resistance, R_h, is approximately $\sim 1/SL_p$ [14], where S is the surface area of the cell membrane and L_p is the membrane permeability. $L_p \sim 10^{-14}$m/s/Pa has been measured for plant cells [15], which have a thick wall like *S. cerevisiae*, and so it is reasonable to guess that these values could be similar. With the known surface area of a yeast cell at $S \sim 3.10^{-11}\mathrm{m}^2$, this leads to an estimate of $P_s \sim J/SL_p \sim 0.25$ MPa for budding yeast. This value is very close to our estimation of P_s. Note that these values are consistent with previous observations in *S.cerevisiae* in which the authors found that $P_t \sim 0.05$ MPa when cells were growing, and $P_t \sim 0.25$ MPa for stationary cells[16]. In the model, we considered that $P_s \sim 0.25$ MPa was constant and did not depend on GIP.

Using all these parameters, we can predict how growth rate, cell number and GIP should vary during growth in confinement. We found that our model predicted our experimental observations remarkably well. This suggests, as discussed in the manuscript, that it is not necessary to invoke a specific biological adaptation through stress pathway signaling to explain the changes in protein production and proliferation that we observe during growth in confinement.

I. Measurement of turgor pressure through laser ablation, AFM and electronic microscopy.

We undertook a series of three experiments to determine cell wall elasticity and thickness, and turgor pressure. Fig. S**??** shows examples of the data.

a. Laser ablation experiments to deflate a cell and to measure a function of turgor pressure and cell wall elasticity. By shooting the border of the cell with a laser, we create a hole in the cell, leading to efflux of cellular contents and deflation owing to loss of turgor and relaxation of cell wall components. Denoting E the cell wall elasticity, ν the Poisson ratio, h the cell wall thickness, $\Delta P = P_t$ the loss of pressure, and R_1 (R_0) the initial (final) radius of the cell, we found:

$$\Delta P = P_t = \frac{R_1 - R_0}{R_1} \frac{h}{R_1} \frac{2E}{1-\nu} \tag{18}$$

Experiments showed a deflation $1 - R_0/R_1 = 0.21 \pm 0.06$ (Fig. S**??**a).

b. Transmission electron microscopy to determine cell wall thickness. High resolution TEM images were acquired to estimate cell wall thickness. While precise measurement is difficult due to the fact that we do not really know where we cut in the cell, averaging over multiple measurements we estimate cell wall thickness to be $h \sim 0.13 \mu m$ (Fig. S**??**b).

c. Atomic force microscopy as an orthogonal method to measure a function of turgor pressure and cell wall elasticity. Recent work from the group of A. Boudaoud investigated the use of AFM as a means to indent a pressurized shell [13]. Two regimes were identified: a small indentation regime where both internal pressure and cell wall elasticity are measured in a coupled way, and a large indentation regime, largely independent of cell wall elasticity, probing internal pressure only. We find that the large indentation regime is impossible to achieve for our cells because the probe usually ruptures the cell wall. Small indentation was however possible, and led to the measurement of the effective spring constant of the cell wall $k \sim 0.15$ N/m (Fig. S**??**c).

Theoretically, for small indentation, the authors derived a relationship between the effective spring constant of the cell wall k and the mechanical properties of the cell (formula 3.6 in their paper). This formula is a function of both P_t and E. Replacing E, we can estimate P_t:

$$P_t = \frac{\sqrt{3\,(1-\nu^2)}}{1-\nu} \frac{R_1 - R_0}{R_1} \frac{k}{\pi h f(\tau)} \tag{19}$$

where:

$$\tau = \frac{\sqrt{(3\,(1-\nu^2)}}{1-\nu} \frac{R_1 - R_0}{R_1} \frac{R_1}{h} \tag{20}$$

and $f(\tau)$

$$f(\tau) = \frac{\sqrt{\tau^2 - 1}}{\mathrm{arctanh}\sqrt{1-\tau^{-2}}} \tag{21}$$

d. Extracting P_t. Using Eqs. 18 and 19 along with the measurement of cell wall thickness h, we were able to extract both the cell wall elasticity (assuming that $\nu \sim 0$) to be $E \sim 2.5$ MPa and the turgor pressure to be $P_t \sim 0.05$ MPa. We used this parameter in the theoretical prediction of experimental data (see above).

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