Supplementary Information

Overlapping and essential roles for molecular and mechanical mechanisms in mycobacterial cell division

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Supplementary Materials

Extended data Movies S1-S3

Extended data Figures S1-S16

Supplementary Table 1

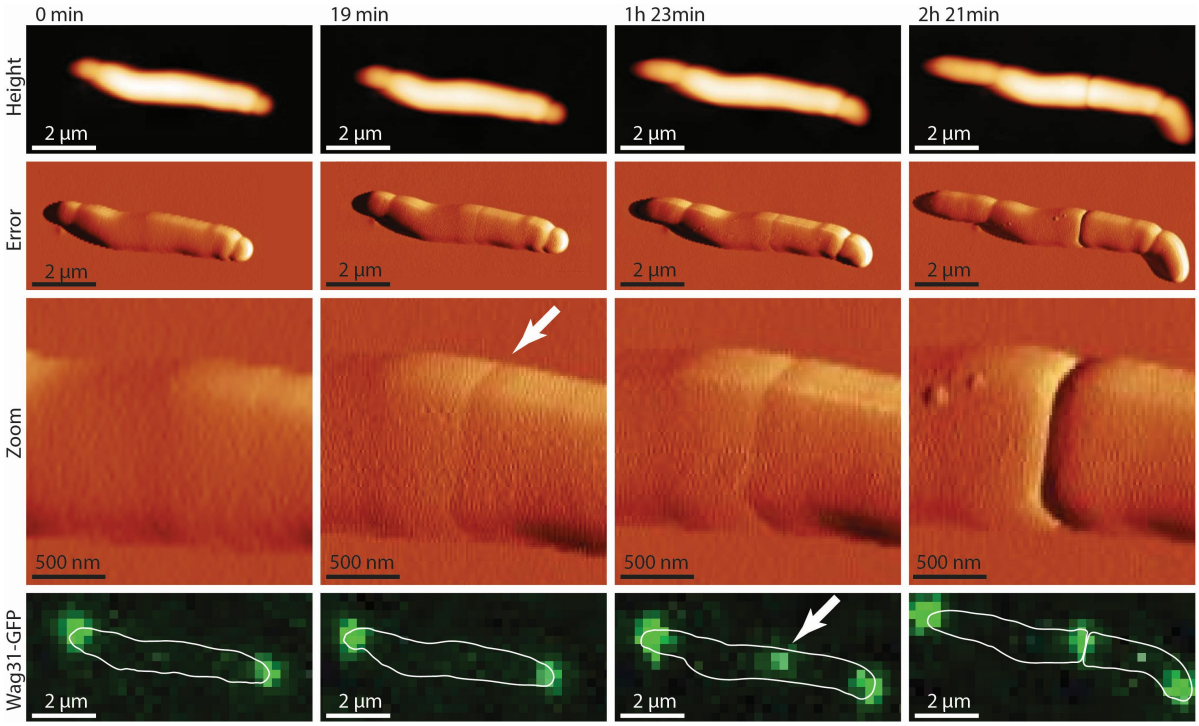
Extended Data Movie Legends:

Extended data Movie S1. Time sequence of *M. smegmatis* cells undergoing abrupt cleavage imaged in PeakForce QNM, stiffness mode with low force set point. Frame rate, 5 minutes. Corresponds to data in Figure 1a.

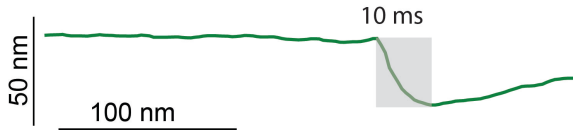
Extended data Movie S2. Time sequence of stiffness increase at the PCF imaged in PeakForce QNM, stiffness mode with higher force set point. Frame rate, 5 minutes. Corresponds to data in Figure 3a.

Extended data Movie S3. Time sequence of alternative mechanism of separation of sibling cells when one sibling cell is deflated by piercing with the AFM cantilever tip. Sibling cells do not undergo rapid cell cleavage; instead, the surviving sibling gradually sheds the deflated sibling. Corresponds to data in Figure 3e.

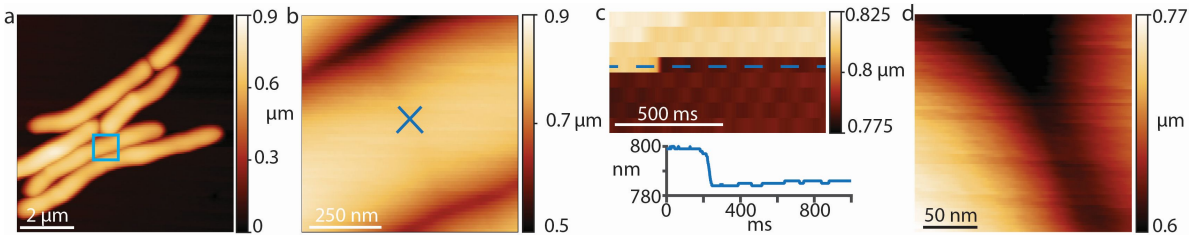
Extended data Figures



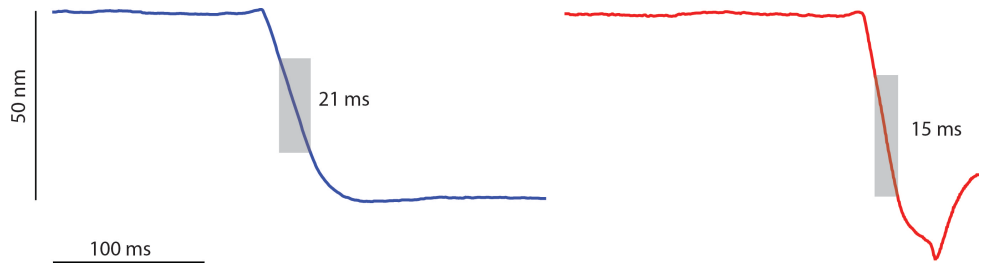
Extended data Figure S1. The pre-cleavage furrow (PCF) precedes the Wag31-GFP cytokinesis marker at the future cell division site. Time sequence of cells expressing Wag31-GFP imaged by correlated AFM and fluorescence microscopy. In this example, the PCF appears at 19 minutes (white arrow). Wag31-GFP, a marker of cytokinesis [1], appears much later and co-localizes with the PCF (arrow at 1 hour 23 minutes) (n=2).



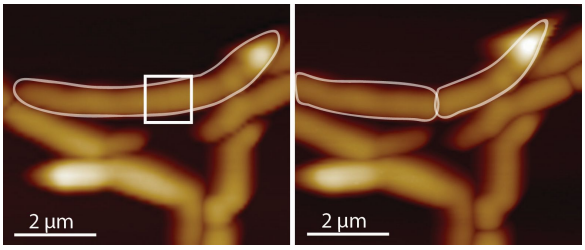
Extended data Figure S2. AFM scan line across the PCF during cleavage. Cleavage of sibling cells was monitored by constantly scanning perpendicularly across the PCF with the AFM cantilever at 10 Hz. A representative example is shown. The cleavage event corresponds to an abrupt drop in the height profile (grey shading). Cleavage is completed within 10 milliseconds and no gradual constriction is observed leading up to this event (flat line).



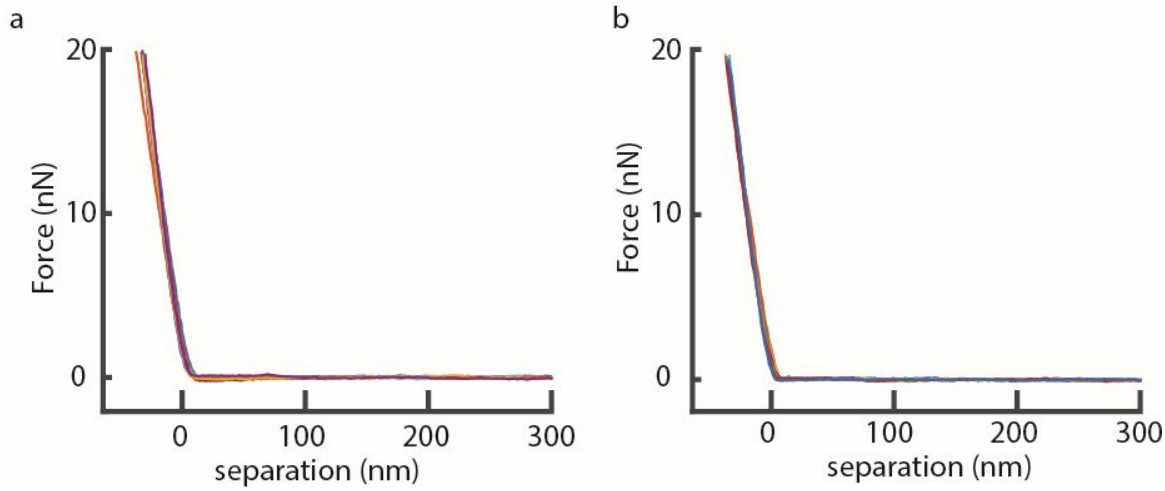
Extended data Figure S3. Height data of millisecond cell cleavage measured at 1 kHz rate (shown in Figure 2e). (a) Overview of a cell initating septation. **(b)** Zoom-in (blue square) to the nascent PCF. The cantilever tip was positioned exactly on the center of the PCF (blue cross 'x') and the height was recorded without scanning the tip laterally at a rate of 1 kHz. **(c)** Height data (upper panel) and height profile (lower panel) at cleavage. **(d)** After the drop in height was observed an image was immediately recorded at the same position to confirm that the drop in height was due to cell cleavage and that the location of the AFM tip remained at the site of cell cleavage.



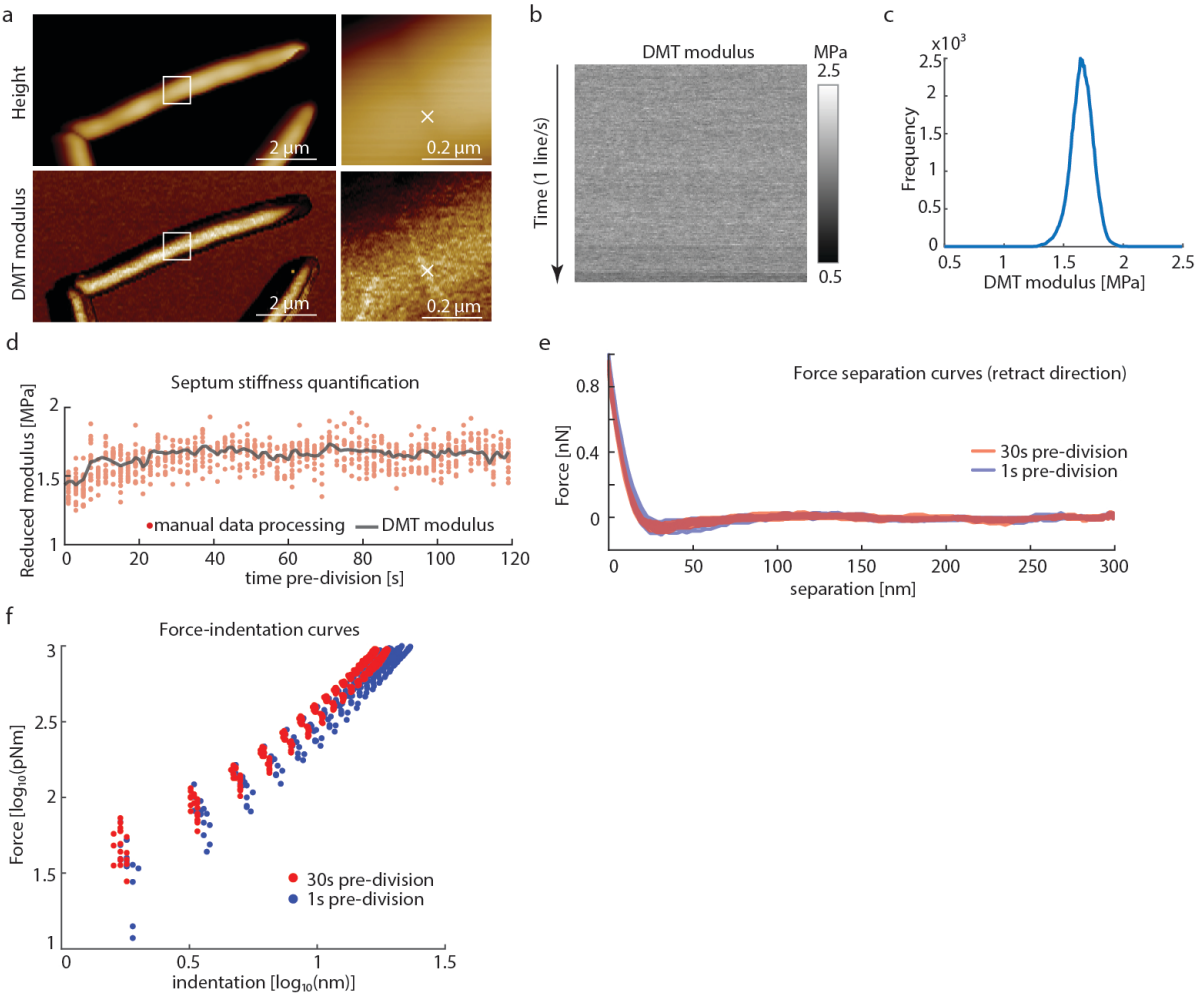
Extended data Figure S4. Cell cleavage measured at 1 millisecond time resolution. The time required for cell cleavage was measured at a 1 kHz rate by constantly measuring the height of a single point on top of the PCF. Two representative examples are shown. The cleavage event corresponds to an abrupt drop in the height profile (grey shading). Cleavage is completed within 21 milliseconds (upper panel) or 15 milliseconds (lower panel) and no gradual constriction is observed leading up to this event (flat line).



Extended data Figure S5. Overview height images of cell before (left) and after (right) cleavage shown in Figure 2f-h. The white square in the image on the left is the region corresponding to the 3D representation in Figure 2f.

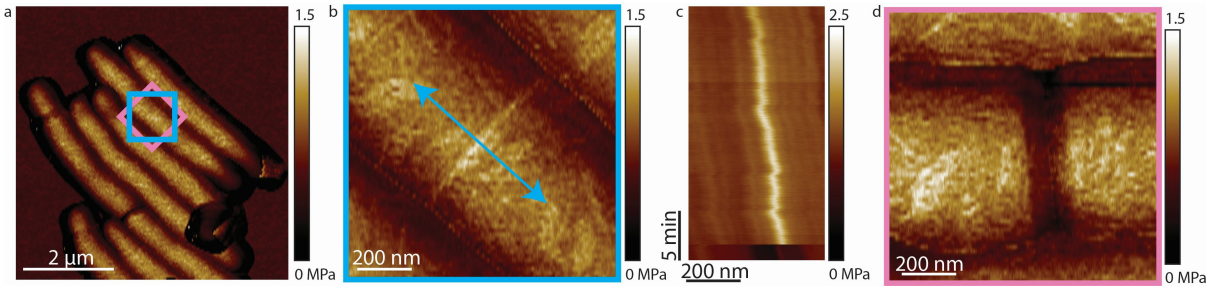


Extended data Figure S6. AFM force curves at the PCF of two representative cells over time. **(a,b)** Force curves on the PCF of two individual cells over time. Compared to measurements in QNM mode, the characteristics of acquiring force curve are significantly different: ~1,000 times lower ramp rate, ~200 times lower indentation velocity, ~20 times higher maximal force applied, and generally larger indentation depth.

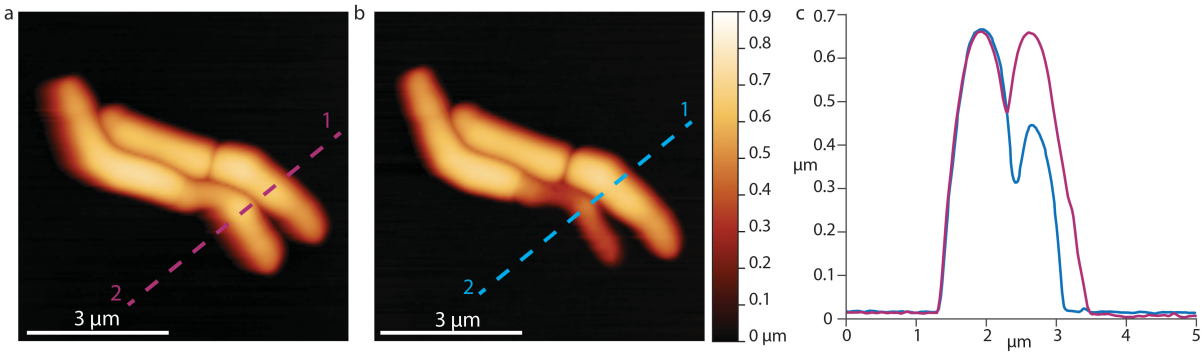


Extended data Figure S7. Comparison of stiffness values obtained from QNM DMT modulus imaging channel and analysis of the individual force-distance curves. To compare the stiffness given by the DMT modulus imaging channel with manual analysis of the recorded force curves, force curves were recorded leading up to a cell cleavage event using QNM mode in peak force capture to record the individual force-distance curves at a ramp rate of 1 kHz and a peak force of 1 nN. **(a)** The cantilever tip was positioned at the center of a PCF (white cross 'x'). **(b)** Force-distance curves were recorded at the same spot on top of the PCF without laterally moving the tip. **(c)** The mean value obtained from the DMT modlus channel was ~1.6 MPa. **(d)** For each line in the DMT modulus image (corresponding to 128 data points in 0.5 seconds) the average stiffness value was calculated

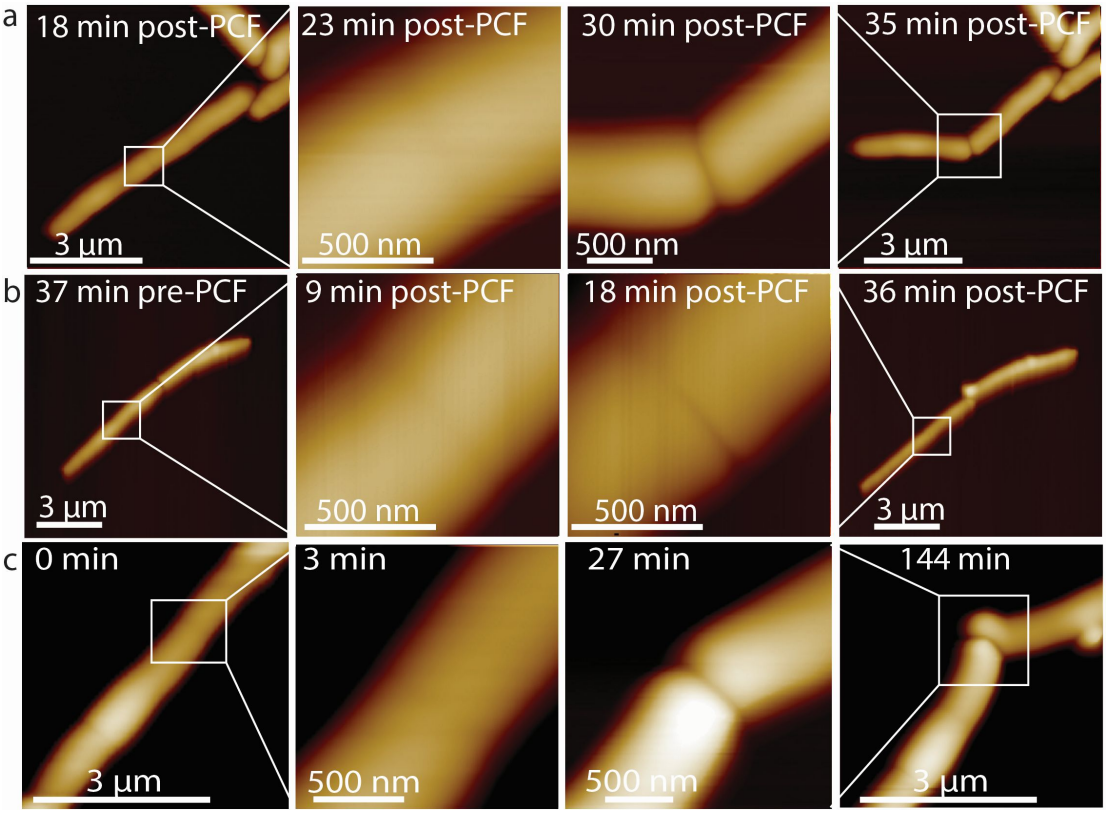
and plotted (black line). Every tenth force curve was then processed manually with Nanoscope Analysis software. Each retract direction of the force curve was corrected to set the baseline to 0 nN, then a moving average filter with a width of 5 units was applied. To extract mechanical property values, a Hertzian fit was used in the range between 4% to 98% of the maximal force (red dots), then 50% of the force curves with the highest r^2 values were plotted. The DMT modulus values extracted from the QNM channel image are in good agreement with manual force-distance analysis. Decrease in stiffness at the septum during the last phase of cell division was observed in the DMT modulus channel and the force curve analysis alike (last ~20 seconds). **(e)** Representative force-separation curves taken at either 30 seconds (red) or 1 second (blue) before cell cleavage. **(f)** To unveil multiple slopes that could potentially derive from different cell wall layers, the same data as in **(e)** were used to plot the slope of the force-separation curves in a log-log plot as has been done by others [2] to discriminate between the contribution of the cell wall and the turgor pressure. However, in these measurements there is no clear distinction that would account for two different linear slopes. The decrease in the stiffness at 1 second (blue) before cleavage compared to the stiffness at 30 seconds (red) before cleavage is clearly discernible in the log-log plot.



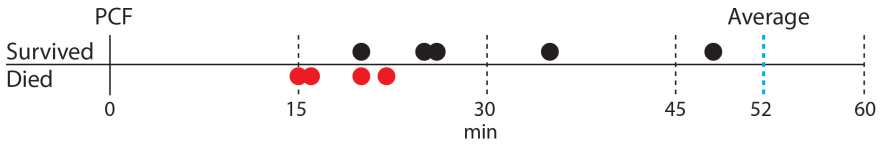
Extended data Figure S8. Stiffness at the PCF measured by repeatedly scanning across the PCF. **(a)** Zoomed-out view of a cell initiating septation. **(b)** Zoom-in on the PCF (blue box in (**a**)). Stiffness evolution over time was measured by repeatedly scanning a line across the PCF at the same location (blue arrow) in QNM mode until a drop in height was observed. **(c)** Kymogram of PCF stiffness over time, proceeding from top to bottom. The abrupt decrease in stiffness at the bottom of the kymogram corresponds to cell cleavage. From these data the stiffness values were extracted as described in the Methods and plotted in Figure 3b. **(d)** Following the abrupt decrease in PCF stiffness recorded in (**c**), zoom-in on the same region of the same cell (pink square in (**a**)) verifed that cleavage had occurred.



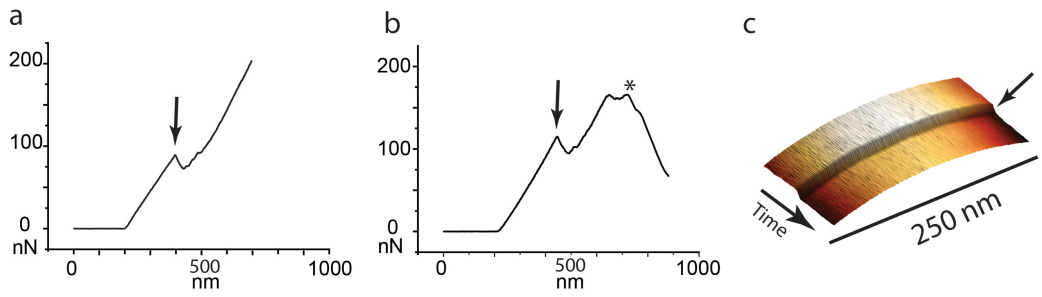
Extended data Figure S9. Height images of cells before and after deflation induced by piercing with a sharp AFM cantilever tip. (a,b) Height images of cells shown in Figure 3e at 0 minutes **(a)** and 1 hour 46 minutes **(b)**. **(c)** Height profiles of cells before (red line) and after (blue line) AFM-mediated piercing show deflation of the cell pierced with the cantilever tip, confirming loss of turgor pressure.



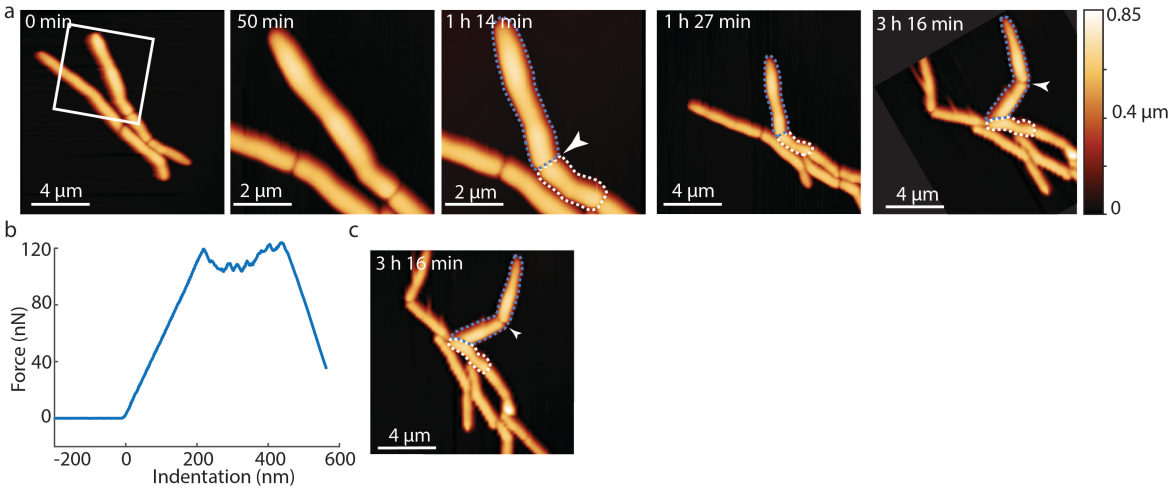
Extended data Figure S10. Height images of cells corresponding to stiffness maps shown in Figure 4.



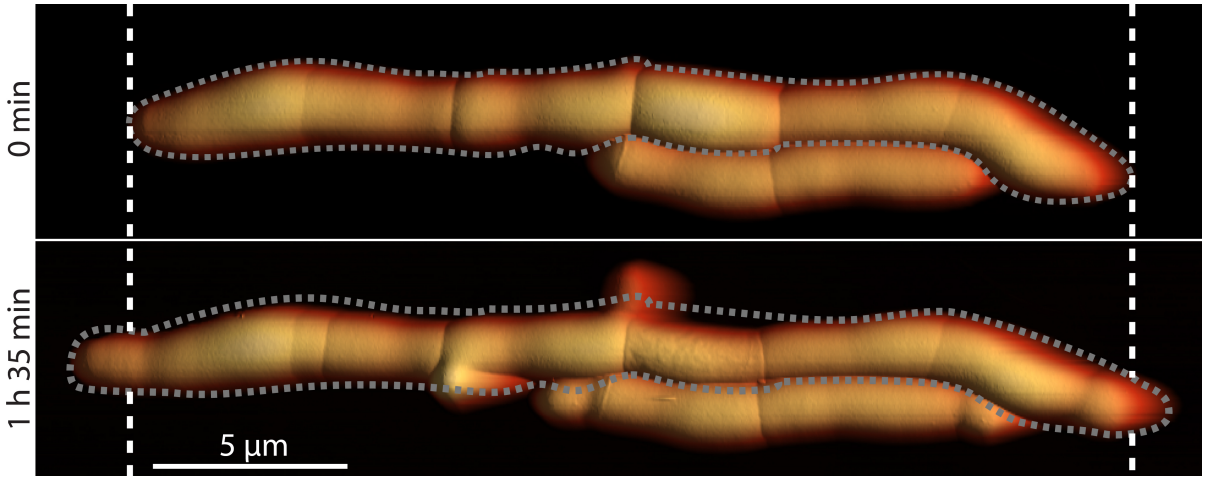
Extended data Figure S11. Cell cleavage can be induced prematurely by increasing the mechanical stress on the PCF. Cell cleavage was induced at different time points after the first appearance of the PCF (0 minutes) by applying a point force on the PCF with the AFM cantilever. In the majority of cases, forced cleavage at early time points resulted in two non-viable daughter cells (red dots), while forced cleavage at later time points resulted in two sibling cells that survived and continued to grow (black dots). The average time of natural cleavage of non-induced cells is indicated by the vertical blue dashed line at 52 minutes (n= 15).



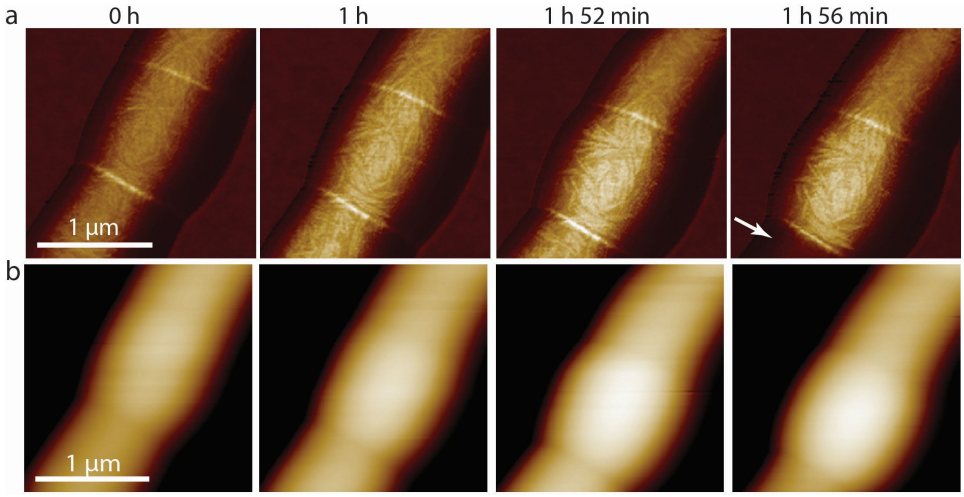
Extended data Figure S12. Premature cleavage can be induced by AFM force curves or AFM scanning along the PCF. Cleavage of cells shown in Figure 4 was induced by applying force with the AFM cantilever, which was done either by recording a force curve (wild-type cells) or by scanning along the septum at an elevated force (RipA-depleted cells). **(a)** Force at the PCF was applied by recording a force curve on the center of the PCF. A sudden drop in the force (black arrow) indicates an induced cleavage event. Subsequent AFM images show newly cleaved poles (see Figure 4a). **(b)** As in **(a)** force was applied at the PCF by recording a force curve. A drop in the force (black arrow) indicates a rupture event of leading to premature partial cleavage as shown in Figure 4b. Subsequently, the increasing force on the forming septum leads to rupture (*) and cell lysis. **(c)** Kymogram of the height while continuously scanning the same line (1 line/second) along the PCF of a RipA-depleted cell at high force. A sudden drop in the height (black arrow) indicates an induced cleavage event. AFM images prior and subsequent to induced cleavage events are shown in Figure 4.



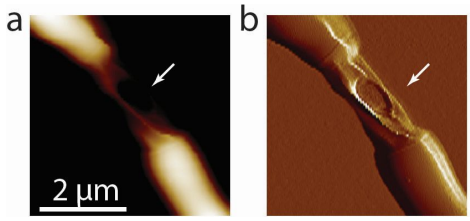
Extended data Figure S13. Cells forced to undergo premature cleavage continue to grow and divide. **(a)** Time lapse AFM height images of cells that continue to grow and divide after cleavage was prematurely induced by application of mechanical stress on the PCF using the AFM cantilever. At 50 minutes, a zoom-in shows the area outlined at 0 minutes (white box). At 1 hour 14 minutes, premature cell cleavage was induced by AFM-mediated application of force on the PCF (white arrow). Between 1 hour 27 minutes and 3 hours 16 minutes, the prematurely cleaved new cell poles elongated and the corresponding sibling cells grew and divided (arrowhead at 3 hours 16 minutes, cropped and rotated to align with previous time points). **(b)** Force curve corresponding to forced cell cleavage at 1 hour 14 minutes. **(c)** Non-cropped and non-rotated image of time-lapse image shown in (a) at 3 hours 16 minutes.



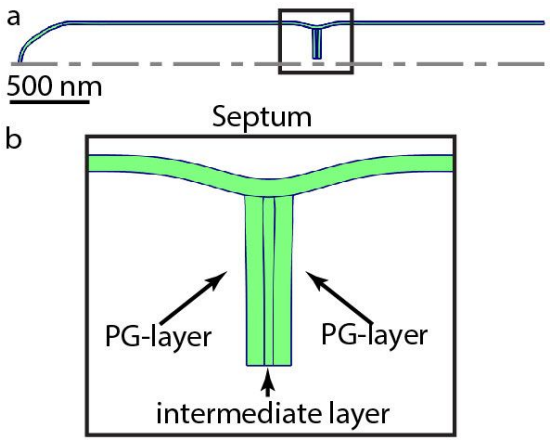
Extended data Figure S14. RipA-depleted cells form chains of cells that grow only at the free cell poles. The two free (outermost) cell poles elongate and new septa continue to form, but failure of septated sibling cells to undergo cleavage, due to depletion of RipA, results in a cell-chaining phenotype. Growth occurs mainly at the free cell poles at the two extremeties of a chain and occasionally at lateral budding locations (cf. 0 minutes and 1 hour 35 minutes). Grey dashed lines indicate the outline of the chain of cells. White dashed lines mark the positions of the free cell poles at 0 minutes.



Extended data Figure S15. Height and stiffness changes of chained RipA-depleted cells. **(a)** Stiffness channel of a centrally located chained and non-elongating RipA-depleted cell over time. While the surface structural features do not change significantly, the measured stiffness at the PCF and the width of the non-elongating central cell increase over time, up to 1 hour 52 minutes (n=3). Upon loss of pressure of the neighboring sibling cell (1 hour 56 minutes, white arrow) the stiffness of the septum and the surviving sibling cell both decrease slightly. **(b)** Corresponding height images of (a). As the central chained cell becomes stiffer it also becomes higher over time.



Extended data Figure S16. Cell wall failure of centrally located cells of RipA-depleted cell chains occurs in between the flanking PCFs. **(a)** AFM height image of a representative chained RipA-depleted cell that died (white arrow). **(b)** AFM error image showing a hole in the middle part of the cell that died.



Extended data Figure S17. Finite element modeling of the PCF in a dividing cell. (a) Global cell geometry with a partially closed septum. The dotted line represents the axis of symmetry. **(b)** Zoom-in on the septum and PCF, showing the top part of the partially closed septum. The peptidoglycan (PG) layer on each side would become the new poles of the two respective daughter cells. These peptidoglycan layers are connected by a soft intermediate layer.

Supplementary Table 1

Similar data as represented in Figure	Experiment description	Total observations	Out of nr. of experiments
1a	PCF appearance before division	15	15
1b	PCF colocalizes with FtsZ	12*	12
1b	PCF site of septum formation	>20*	>20
S1	PCF site of localization of wag31	>10*	>10
1c	division scar at site of PCF	>15	>15
2	rapid (millisecond) division	>22	>22
3a-b	significant and steady increase of stiffness	22	22
3c-d	stiffness at PCF and sister cell with sorbitol/water	1	1
3e	pierce one sibling cell after cytokinesis	2	2
4a	induced cell division with viable cells	11	11
4b	induced cell division with dead cells	5	5
S15	center RipA-depleted cells become stiffer & wider	3	3
4c	Induced cleavage RipA-depleted cells	4	4
4c	free RipA-depleted cells grow out after induced cleavage	2	2

References:

1 Santi, I., Dhar, N., Bousbaine, D., Wakamoto, Y. & McKinney, J. D. Single-cell dynamics of the chromosome replication and cell division cycles in mycobacteria. *Nat Commun* **4**, doi:10.1038/ncomms3470 (2013).

2 Bailey, R. G. *et al.* The interplay between cell wall mechanical properties and the cell cycle in Staphylococcus aureus. *Biophys J* **107**, doi:10.1016/j.bpj.2014.10.036 (2014).