Peptidoglycan synthesis drives an FtsZ-treadmilling-independent step of cytokinesis

João M. Monteiro1*, Ana R. Pereira1*, Nathalie T. Reichmann1, Bruno M. Saraiva1, Pedro B. Fernandes1, Helena Veiga1, Andreia C. Tavares1, Margarida Santos1, Maria T. Ferreira1, Vânia Macário1, Michael S. VanNieuwenhze2, Sérgio R. Filipe1 & Mariana G. Pinho1

Peptidoglycan is the main component of the bacterial wall and protects cells from the mechanical stress that results from high intracellular turgor. Peptidoglycan biosynthesis is very similar in all bacteria; bacterial shapes are therefore mainly determined by the spatial and temporal regulation of peptidoglycan synthesis rather than by the chemical composition of peptidoglycan. The form of rod-shaped bacteria, such as Bacillus subtilis or Escherichia coli, is generated by the action of two peptidoglycan synthesis machineries that act at the septum and at the lateral wall in processes coordinated by the cytoskeletal proteins FtsZ and MreB, respectively1,2. The tubulin homologue FtsZ is the first protein recruited to the division site, where it assembles in filaments—forming the Z ring—that undergo treadmilling and recruit later divisome proteins3,4. The rate of treadmilling in B. subtilis controls the rates of both peptidoglycan synthesis and cell division5. The actin homologue MreB forms discrete patches that move circumferentially around the cell in tracks perpendicular to the long axis of the cell, and organize the insertion of new cell wall during elongation5,6. Cocci such as Staphylococcus aureus possess only one type of peptidoglycan synthesis machinery7,8, which is diverted from the cell periphery to the septum in preparation for division9. The molecular cue that coordinates this transition has remained elusive. Here we investigate the localization of S. aureus peptidoglycan biosynthesis proteins and show that the recruitment of the putative lipid II flippase MurJ to the septum, by the DivIB–DivIC–FtsL complex, drives peptidoglycan incorporation to the midcell. MurJ recruitment corresponds to a turning point in cytokinesis, which is slow and dependent on FtsZ treadmilling before MurJ arrival but becomes faster and independent of FtsZ treadmilling after peptidoglycan synthesis activity is directed to the septum, where it provides additional force for cell envelope constriction.

In cocci, one possible molecular cue determining the shift of peptidoglycan (PG) synthesis from the cell periphery to the septum could be the recruitment to the midcell of a key PG biosynthesis protein, concomitant with divisome assembly. We therefore investigated the localization of most of the S. aureus PG synthesis proteins in the background of the methicillin-resistant S. aureus (MRSA) strain COL (Fig. 1 and Supplementary Table 1). All fluorescent fusions were functional (Supplementary Table 1) and expressed from their native locus under the control of their native promoter as the sole copy of the gene in question. The only septal proteins for which virtually no signal could be observed were the synthesis enzymes to be highly enriched in the septal region of dividing cells. However, MraY, MurG and the FemXAB proteins were evenly distributed throughout the membrane (including the septum), which suggests that the key step for spatial regulation of PG synthesis was not the synthesis of lipids I or II (Fig. 1b, c). MurJ, FtsW and PBPI were the only septal proteins for which virtually no signal could be expected in the peripheral membrane during septum synthesis (Fig. 1b, c), and therefore represented good potential candidates as catalysts for the first step of PG synthesis that is diverted to the septal region. MurJ is a member of the multidrug–oligosaccharidyl–lipid–polysaccharide (MOP) exporter superfamily and has previously been suggested to be the lipid II flippase in E. coli10. In S. aureus, the essential gene SAV1754 (also known as SACOL1804) has previously been reported to be a functional MurJ orthologue11. FtsW is a member of the sporeulation, elongation, division and synthesis (SEDS) protein family, also suggested to be a lipid II flippase12. However, SEDS proteins have more recently been shown to be PG transglycosylases that probably function together with a cognate penicillin-binding protein (PPB) during PG polymerization13,14. S. aureus encodes two SEDS proteins, SACOL112 and SACOL2075, which are similar to the B. subtilis proteins FtsW and RodA, respectively. PBPI is a transpeptidase that crosslinks PG glycan strands15.

To clarify which protein or set of proteins was responsible for directing PG synthesis to the septum, newly synthesized PG was labelled with the fluorescent α-amino acid HCC-amino-d-alanine (HADA) which is specifically incorporated into PG16, in an S. aureus strain expressing both FtsW–mCherry (which co-localizes with PBPI, see later) and MurJ–sGFP. HADA incorporation appeared to co-localize with both proteins in cells in phase 1 of the cell cycle and in most phase 2 cells (Fig. 2a; see Fig. 1d for cell-cycle phases). However, MurJ and HADA septal co-localization was more frequent than FtsW and HADA septal co-localization (88% versus 70% of cells, n = 200), as cells with septal FtsW but peripheral MurJ had peripheral HADA incorporation (see asterisks in Fig. 2a). This suggested that septal PG synthesis was dependent on the presence of MurJ. If this was the case, preventing MurJ recruitment to the midcell should abolish septal PG synthesis. We therefore investigated the mechanism of MurJ localization so that we could selectively prevent its septal recruitment while maintaining correct FtsW and PBPI septal localization. For this purpose, we determined the timing of MurJ arrival to the septum, because the localization of divisome proteins is often dependent on the presence of early localizing proteins17,18.

In B. subtilis, divisome assembly is a two-step process: proteins such as FtsA, ZapA and EzrA arrive very early and concomitantly with FtsZ, and are followed after a time delay by a second group of proteins including the DivIB–DivIC–FtsL sub-complex19. We therefore compared S. aureus PBPI, FtsW and MurJ localization with that of

1Bacterial Cell Biology, Instituto de Tecnologia Química e Biológica António Xavier, Universidade Nova de Lisboa, Oeiras, Portugal. 2Department of Chemistry, Indiana University, Bloomington, Indiana, USA. 3Instituto de Tecnologia Química e Biológica António Xavier and Faculdade de Ciências e Tecnologia, Universidade Nova de Lisboa, Oeiras, Portugal. *These authors contributed equally to this work.

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the early divisome protein FtsZ and the later divisome protein DivIB (Fig. 2b). Co-localization between each protein and FtsZ was determined by measuring Pearson’s correlation coefficient (PCC) for the fluorescence signals in the two channels in cells that showed FtsZ midcell localization. We reasoned that proteins arriving to the septum simultaneously with FtsZ should have a PCC close to 1 and that this value should decrease for later divisome proteins. We constructed an S. aureus strain that co-expressed FtsZ–CFP and FtsZ–mCherry as a positive control. Co-localization results indicated that DivIB arrives at the divisome later than FtsZ, as expected, and at approximately the same time as PBP1 and FtsW, whereas MurJ arrives later than DivIB, PBP1 and FtsW (Fig. 2c); this result is unlikely to be affected by the nature of the fluorescent tags (Extended Data Fig. 1). Consistent with these timings, in 20% of the cells (n = 200) of an S. aureus strain that expressed both YFP–DivIB and MurJ–mCherry, YFP–DivIB was already localized at the septum whereas MurJ–mCherry had not yet arrived (insets in Fig. 2d). This raised the possibility that MurJ septal recruitment is dependent on the presence of the DivIB–DivIC–FtsL sub-complex. We therefore depleted expression of each of these three proteins using antisense RNA fragments (Supplementary Table 2) and depletion co-localization of FtsZ–CFP and MurJ–mCherry (Fig. 3a, b), but not of FtsZ and either FtsW or PBP1 (Extended Data Fig. 3), consistent with their earlier recruitment to the divisome.

Having developed a tool to specifically delocalize MurJ by depleting FtsL while at the same time maintaining correct PBP1 and FtsW localization, we were able to determine that new PG, labelled by a short pulse of HADA, was only incorporated at the midcell if MurJ–sGFP was already localized at the septum whereas MurJ–mCherry had not yet arrived (Fig. 3c). Finaly, we showed that inhibiting MurJ activity using DMPI, a MurJ inhibitor12, that does not prevent the recruitment of MurJ to the divisome (Extended Data Fig. 2). Depletion of DivIB, DivIC or FtsL reduced septal co-localization of FtsZ–CFP and MurJ–mCherry (Fig. 3a, b), but not of FtsZ and either FtsW or PBP1 (Extended Data Fig. 3), consistent with their earlier recruitment to the divisome.

Taken together, these data indicate that recruitment of MurJ to the divisome by the DivIB–DivIC–FtsL complex is likely to be the molecular cue that directs PG synthesis specifically to the septum.
During division. Therefore, we would expect MurJ to be essential for the transition from phase 1 to phase 2 during the cell cycle (that is, for initiation of septum synthesis). We treated COL wild-type cells with DMPI, PC190723, oxacillin, dimethyl sulfoxide (DMSO) and therefore to control the rate of PG synthesis during septum formation in B. subtilis and therefore to control the rate of cell division. Consequently, one would expect that the addition of PC190723 would prevent phase 2 cells that were halfway through the process of septum synthesis from completing this process. As this was not the case we considered whether the redirection of PG synthesis to the septum—which is driven by septal recruitment of MurJ—could provide the constrictive driving force for cytokinesis, as has previously been suggested for E. coli23,24, and result in the closing of the septum independent of FtsZ treadmilling. To visualize the closing of the septum and determine whether FtsZ treadmilling occurs in S. aureus, we introduced an FtsZ sandwich fusion to superfast GFP (sGFP), in which sgfp was inserted in the ftsZ coding sequence between codons 55 and 56 (FtsZ^55–56sGFP; see Methods) in the background of a strain that expressed native FtsZ, and then followed the dynamics of Z-ring formation and constriction. We were able to observe the movement of FtsZ^55–56sGFP, which as expected was inhibited by PC190723 (Fig. 4 and Supplementary Video 1). However, Z-ring constriction continued in many cells treated with PC190723, consistent with the fact that PC190723-treated cells could complete phase 2 of the cell cycle (Fig. 3e). When we followed Z-ring constriction in untreated cells we observed biphasic cytokinesis, with a first step immediately after Z-ring assembly during which the divisome barely constricts, followed by a second step with a higher rate of Z-ring constriction (Fig. 5a, Extended Data Fig. 6a and Supplementary Video 2). The addition of PC190723 blocked the constriction of large Z rings, presumably in the first step of cytokinesis, but not of smaller Z rings (Fig. 5a, b, Extended Data Fig. 6a and Supplementary Video 3). This shows that only Z rings in the first step of cytokinesis required treadmilling activity for constriction. To confirm these results, we used a functional, fluorescent derivative of the divisome protein EzrA as a proxy for FtsZ localization, because EzrA interacts directly with FtsZ25,26. Similar to our observations for FtsZ, EzrA treadmilling was inhibited by PC190723, EzrA rings underwent biphasic constriction and the second and faster step of cytokinesis was insensitive to PC190723 (Extended Data Fig. 7).

It is possible that the transition between the first and the second step of Z-ring constriction corresponds to the start of substantial PG synthesis, and therefore to control the rate of PG synthesis during septum formation in B. subtilis and therefore to control the rate of cell division. Consequently, one would expect that the addition of PC190723 would prevent phase 2 cells that were halfway through the process of septum synthesis from completing this process. As this was not the case we considered whether the redirection of PG synthesis to the septum—which is driven by septal recruitment of MurJ—could provide the constrictive driving force for cytokinesis, as has previously been suggested for E. coli23,24, and result in the closing of the septum independent of FtsZ treadmilling. To visualize the closing of the septum and determine whether FtsZ treadmilling occurs in S. aureus, we introduced an FtsZ sandwich fusion to superfast GFP (sGFP), in which sgfp was inserted in the ftsZ coding sequence between codons 55 and 56 (FtsZ^55–56sGFP; see Methods) in the background of a strain that expressed native FtsZ, and then followed the dynamics of Z-ring formation and constriction. We were able to observe the movement of FtsZ^55–56sGFP, which as expected was inhibited by PC190723 (Fig. 4 and Supplementary Video 1). However, Z-ring constriction continued in many cells treated with PC190723, consistent with the fact that PC190723-treated cells could complete phase 2 of the cell cycle (Fig. 3e). When we followed Z-ring constriction in untreated cells we observed biphasic cytokinesis, with a first step immediately after Z-ring assembly during which the divisome barely constricts, followed by a second step with a higher rate of Z-ring constriction (Fig. 5a, Extended Data Fig. 6a and Supplementary Video 2). The addition of PC190723 blocked the constriction of large Z rings, presumably in the first step of cytokinesis, but not of smaller Z rings (Fig. 5a, b, Extended Data Fig. 6a and Supplementary Video 3). This shows that only Z rings in the first step of cytokinesis required treadmilling activity for constriction. To confirm these results, we used a functional, fluorescent derivative of the divisome protein EzrA as a proxy for FtsZ localization, because EzrA interacts directly with FtsZ25,26. Similar to our observations for FtsZ, EzrA treadmilling was inhibited by PC190723, EzrA rings underwent biphasic constriction and the second and faster step of cytokinesis was insensitive to PC190723 (Extended Data Fig. 7).

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Figure 5 | PG synthesis provides force for septum constriction. a, Time-lapse images and corresponding kymographs of FtsZ55–56sGFP rings in the absence (control) or presence (+PC) of the FtsZ inhibitor PC190723. Kymographs were obtained by drawing a line (example shown in red) across the cell for each time point. Arrowheads point to the transition from the first (no or slow constriction) to the second (fast constriction) step of cytokinesis. b, Percentage of FtsZ55–56sGFP rings of different diameters that constricted in the presence of PC190723 (n = 248).

c, Kymographs of FtsZ55–56sGFP, FtsW–sGFP and MurJ–sGFP larger (left) and smaller (right) rings. PC190723 abolished only the constriction of larger rings. d, Graphs showing diameter of FtsZ–mCherry and MurJ–sGFP rings (left) or FtsZ–mCherry and FtsW–sGFP rings (right) in single cells during cytokinesis. e, Percentage of FtsZ55–56sGFP, FtsW–sGFP and MurJ–sGFP rings that constricted in the presence of PC190723 (FtsZ, n = 254; FtsW, n = 176; MurJ) = 188). Data in a and c are representative of three biological replicates. Scale bars, 0.5 μm.

PG synthesis activity that results from MurJ recruitment. Consistent with this hypothesis, the divisome rings that contained MurJ did not display the first step of constriction and were insensitive to PC190723 (Fig. 5c, e and Extended Data Figs 6b, 8). Furthermore, the arrival of MurJ at the septum coincided with the initiation of fast constriction (Fig. 5d and Extended Data Fig. 9a). By contrast, rings that contained the earlier divisome protein FtsW paralleled the biphasic behaviour of the Z ring (Fig. 5c, d, Extended Data Figs 8, 9b) and were susceptible to inhibition by PC190723, presumably during the initial stages of cytokinesis (Fig. 5e). The FtsZ inhibitor PC190723 blocked the constriction of Z rings only at initial stages, and the addition of the MurJ inhibitor DMP1 blocked ring constriction at all stages (Extended Data Fig. 6a).

We propose a model (Extended Data Fig. 10) in which the S. aureus PG synthesis machinery continuously incorporates PG at the periphery of the cell during initial stages of the cell cycle. In preparation for division, and following Z-ring assembly, the DivIB–DivIC–FtsL complex recruits MurJ to the divisome, which ensures that translocation of lipid II occurs exclusively at the midcell. Substrate affinity then diverts the major PG synthase, PBPs2, from the periphery to the midcell where together with other PG synthesis enzymes it incorporates lipid II into the growing PG network. This mechanism means that an additional dedicated multi-protein machinery is unnecessary, and represents a mode of controlling PG synthesis in two different locations in the absence of an MreB-like cytoskeleton.

After the initiation of large-scale PG synthesis activity at the leading edge of the constricting septum that follows MurJ recruitment, the FtsZ inhibitor PC190723, which inhibits FtsZ treadmilling, no longer prevents cytokinesis. Nevertheless, FtsZ treadmilling is likely to have a role in the organization of septum synthesis, because approximately 15% of the Z rings that were able to constrict in the presence of PC190723 did so defectively, in a similar fashion to E. coli FtsZ mutants that are impaired in GTPase activity4.

Our data may reconcile two models of the origin of the force required for cytokinesis to occur. In one model, this force has been proposed to be derived from FtsZ, either from the chemical energy of GTP hydrolysis that could promote bending of the FtsZ polymers or from the propensity for FtsZ filaments to bundle, which could result in condensation of the Z ring24,28. Alternatively, PG synthesis has previously been suggested to provide the force for cytokinesis23,24. We propose that cytokinesis occurs in two steps: an initial slow step, which is dependent on FtsZ treadmilling, for which FtsZ may be the driving force and that may be responsible for the initial invagination of the cell membrane, followed by a second and faster step for which PG synthesis provides the driving force.

Online Content Methods, along with any additional Extended Data display items and Source Data, are available in the online version of the paper; references unique to these sections appear only in the online paper.

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Supplementary Information is available in the online version of the paper.

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Author Contributions J.M.M., A.R.P., N.T.R. and M.G.P. designed the research. J.M.M. and A.R.P. performed all experiments with the exception of the effect of antibiotics on the cell cycle which was performed by P.B.F. B.M.S. developed software for image analysis. J.M.M., A.R.P., N.T.R., S.R.F. and M.G.P. analysed the overall data. B.M.S. analysed microscopy data quantified by eHooke software. P.B.F. analysed cell-cycle data. J.M.M., A.R.P., N.T.R. and M.G.P. wrote the manuscript.

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**METHODS**

No statistical methods were used to predetermine sample size. The experiments were not randomized and investigators were not blinded to allocation during experiments and outcome assessments.

**Bacterial growth conditions.** Strains and plasmids used in this study are listed in Supplementary Table 3. *S. aureus* strains were grown in tryptic soy broth (TSB, Difco) at 200 r.p.m with aeration at 37 °C or on tryptic soy agar (TSA, Difco) at 30 or 37 °C. *E. coli* strains were grown in Luria–Bertani broth (Difco) with aeration, or in Luria–Bertani agar (Difco), at 30 or 37 °C. When necessary, the antibiotics ampicillin (100 μg ml⁻¹), erythromycin (10 μg ml⁻¹), kanamycin (50 μg ml⁻¹), neomycin (50 μg ml⁻¹) or chloramphenicol (30 μg ml⁻¹) were added to the media. 5-Bromo-4-chloro-3-indolyl-β-d-galactopyranoside (X-gal, Apollo Scientific) was used at 100 μg ml⁻¹. Unless stated otherwise, isopropyl-β-d-1-thiogalactopyranoside (IPTG, Apollo Scientific) was used at 0.1 mM to induce expression of constructs under the control of the Psac promoter. Cadmium chloride (Sigma-Aldrich) was used at 0.1 μM when required, to induce expression of constructs under the control of the Pcad promoter.

**Construction of *S. aureus* fluorescent strains.** Cloning of fluorescent fusions in *S. aureus* was done using the following general strategy: plasmids were propagated in *E. coli* strains DC10B or DH5α and purified from overnight cultures supplemented with the relevant antibiotics. Plasmids were then introduced into electrocompetent *S. aureus* RN4200 cells as previously described34 and transduced into COL using phage 80α. Constructs were confirmed by PCR and by sequencing of the amplified fragment.

The ColMurB-GFP, ColMurD-GFP, ColMurF-GFP, ColFemB-GFP and ColSGezaA-GFP strains were constructed using the pSG5082 vector. In brief, DNA fragments with approximately 500 bp spanning the 3′ ends (minus the stop codons) of the *murB*, *murD*, *murF*, *femB* and *eca* genes from COL were amplified using the primer pairs murBg_P1–murBg_P2, murDg_P1–murDg_P2, murFg_P1–murFg_P2, femBg_P1–femBg_P2 and ecaP8Kpn–ecaAP9Xho, respectively (Supplementary Table 4). Fragments were digested with KpnI and XhoI restriction enzymes and cloned into pSG5082 upstream of and in frame with gfpNmat2, producing the plasmids pSG-murB, pSG-murD, pSG-murF, pSG-femB and pSG-ecaA. These plasmids were then electroporated into RN4200, where they integrated in the genome by a single homologous recombination event and subsequently transduced to COL. Resulting strains contained the corresponding fluorescent fusions in the native locus of each gene under the control of its native promoter, followed by the pSG5082 backbone and a truncated copy of the gene.

To construct ColFemX-sGFP, we used essentially the same strategy, except that the pFAST3 vector was used instead of pSG5082. A second DNA fragment that contained the full *femX* gene without its stop codon and a five-amino-acid linker was amplified by PCR from COL DNA using the primers femXg_P5 and femXg_P6. The two fragments were joined by overlap PCR and digested with Sall and XhoI and cloned into pCNX downstream of the Pspac promoter, giving plasmid pCN-xfemX-sGFP.

The strain ColFtsZ55–56-sGFP was constructed using the pCN51 replicative plasmid to express an FtsZ–sGFP sandwich fusion. In brief, three DNA fragments (F1, F2 and F3) with overhangs were amplified to construct a sGFP fusion inserted within the *ftsZ* coding sequence between codons 55 and 56,66 flanked by 10-amino-acid linkers (Gly-Gly-Gly-Gly-Ser × 2), F1, which contained a ribosome binding site and the first 165 bp of *ftsZ*, was amplified from COL DNA using the primers ftsZswgfp_pCNX_P1 and ftsZswgfp_pCNX_P2. F2, which contained sGFP flanked by linker sequences, was amplified from pTRC99a-P7 using the primers ftsZswgfp_pCNX_P3 and ftsZswgfp_pCNX_P4. F3 contained the remaining 1,008 bp of *ftsZ* (from nucleotide 166 onwards), was amplified from COL DNA using the primers ftsZswgfp_pCNX_P5 and ftsZswgfp_pCNX_P6. The three fragments were joined by overlap PCR, digested with BamHI and EcoRI, and cloned into pCNX to give pCN-ftsZ55–56-sGFP, which was then transduced into COL. The resulting strain was named ColFtsZ55–56-sGFP.

The strain ColMrA-sYF was constructed using the pCN51 replicative plasmid to express an MraY–sGFP sandwich fusion. In brief, three DNA fragments (F1, F2 and F3) with overhangs were amplified to construct a fusion with sGFP inserted within the *mraY* coding sequence, between codons 220 and 221. F1, which contained a ribosome binding site and the first 660 bp of *mraY*, was amplified from COL DNA using the primers mraYg_P1 and mraYg_P2, F2, which contained the stop codon and a 3′ terminal overhang was amplified from pTRC99a-P7 using the primers mraYg_P3 and mraYg_P4. F3, which contained the last 306 bp of *mraY*, was amplified from COL using the primers mraYg_P5 and mraYg_P6. The three fragments were then joined by overlap PCR, digested with Sall and cloned into pCN51, which produced pCN-mraY-sGFP.

The strains ColWZ and ColJZ were constructed by transducing the plasmids pMAD-ftsWsgfp and pMAD-murJsgfp, respectively, into BCBAJ020. ColP12 was constructed by transducing pBCB13-ftsZmch into COLsGFP-PBP1. The strains ColWgZm and ColJgZm were constructed by transducing the plasmids pMAD-ftsWsgfp and pMAD-murJsgfp, respectively, into ColFtsZ-mCherry. The strain ColW was obtained by transducing pMAD-ftsWsgfp from ColFtsZ-mCherry to COL. In each case, allelic replacement was performed as described earlier.

To construct CoFLZ, a fusion was constructed from the genomic DNA of ColFtsZ-mCherry with the primers *ftsZmCherry_pCNX_P1 and *ftsZmCherry_pCNX_P2, digested with BamHI and EcoRI, and cloned into pCNX downstream of the Pcad promoter, giving plasmid pCN-ftsZmCherry. This plasmid was then electroporated into RN4200 and transduced to BCBAJ020, which produced strain CoFLZ.

To study co-localization between DivIB and FtsZ or MraY, an yfp–divIB fusion was constructed and cloned into pCNX. In brief, a DNA fragment that contained yfp minus the stop codon and a 3′ terminal overhang was amplified from pMUTINYFPKan37 with the primers ydivIB_pCNX_P1 and ydivIB_pCNX_P2. A second DNA fragment containing the full divIB gene with a 5′ overhang was amplified from COL DNA with the primers ydivIB_P3 and ydivIB_P4. The two fragments were then joined by overlap PCR, digested with Sall and KpnI, and cloned into pCNX downstream of Pcad, which produced the plasmid pCN-yfpDivIB. This plasmid was transduced to BCBAJ020 and ColFtsZ-mCherry to produce the strains CoZIB and CoJIB, respectively.
Construction of $S. aureus$ strains containing antisense RNA vectors. To construct strains carrying antisense RNA vectors, 250-bp fragments of divIB or divIC genes were amplified from COL DNA with the primer pairs ASDivIB_P1 and ASDivIB_P2, and ASDivIC_P1 and AS_DivIC_P2, respectively, then digested with EcoRI and BamHI and cloned in an antisense direction—relative to the xylose-inducible TSX promoter—into pEPEAS to produce pAS-DivIB and pAS-DivIC. These plasmids were then transduced into ColJ to produce ColJpAS-DivIB and ColJpAS-DivIC, respectively. Additionally, phage lysates were obtained from the field strains$^{24}$ carrying the antisense RNA pEPEAS vectors pAS-022 and pAS-185, which target ftsA and ftsL, respectively. pAS-022 was transduced to ColWZ and ColP1Z to produce the strains ColWZpAS-FtsA and ColP1ZpAS-FtsA, respectively. pAS-185 was transduced to ColIZ, ColWZ, ColP1Z and ColWJ to produce the strains ColZpAS-FtsL, ColWZpAS-FtsL, ColP1ZpAS-FtsL and ColWJpAS-FtsL, respectively. Control strains for these experiments were obtained by transducing the empty vector pEPEAS into ColIZ, ColWZ, ColP1Z and ColWJ to produce the strains ColZpEPSEPs, ColWZpEPSEPs, ColP1ZpEPSEPs and ColWJpEPSEPs, respectively.

Growth curves of $S. aureus$ strains. Overnight cultures of COL strains that encoded fluorescent derivatives of PG synthesis enzymes were back-diluted to an OD$_{600nm}$ of 0.02 in TSB and grown at 37°C for 11 h with OD$_{600nm}$ measurements taken every hour. Doubling times were calculated for each strain during its exponential growth phase.

Minimum inhibitory concentration assays. Minimum inhibitory concentrations of relevant antimicrobial compounds were determined by broth microdilution in sterile 96-well plates. The medium used was TSB that contained a series of twofold dilutions of each compound. Cultures of $S. aureus$ strains and mutants were added at a final density of approximately $5 \times 10^8$ CFU ml$^{-1}$ to each well. Wells were reserved in each plate for sterility control (no cells added) and cell viability (no compound added). Plates were aerobically incubated at 37°C. Examination of plates were assessed visually after 24 and 48 h. All assays were done in triplicate.

Western blotting. The $S. aureus$ strains ColZpEPSEPs, ColZpEPSEPs-DivIB and ColZpEPSEPs-DivIC were grown overnight, back-diluted to 1:200 in fresh TSB and incubated at 37°C until they reached an OD$_{600nm}$ of 0.2. At this point, xylose was added to the medium at 4% to enable the expression of the antisense RNA fragments. After 1 h of antisense expression, cells were collected and broken with glass beads in a FastPrep FP120 cell disrupter (Thermo Electron). Samples were centrifuged to remove unbroken cells and debris, and the total protein content of the clarified lysates was determined using the Bradford method and bovine serum albumin as a standard (BCA Protein Assay Kit, Pierce). Equal volumes of total protein from each sample were separated on 10% SDS–PAGE at 80 V and then transferred to Hybond-P PVDF membrane (GE Healthcare) using a semi-dry transfer cell (Biorad), according to standard western blotting techniques. Membranes were cut to separate the PB2A region from the DivIB or DivIC region. DivIB and DivIC proteins were detected using specific polyclonal antibodies at 1:5000 and 1:10000 dilutions, respectively. PB2A was detected using the antibody from a Slides MRSA detection kit (Biomerieux) at 1:500 dilution. Protein bands were visualized using the ECL Prime Detection Reagents (GE Healthcare), according to the manufacturer’s instructions. The relative amounts of each protein band were quantified using Fiji software.$^{39}$

Imaging of FtsZ$^{55–56}$sGFP treadmilling by SIM. For time-lapse experiments, all cultures were grown overnight in TSB. For ColFtsZ$^{55–56}$sGFP and ColpSGEzrA-GFP, the medium was supplemented with kanamycin (150 μg ml$^{-1}$) and erythromycin (10 μg ml$^{-1}$), respectively. Cultures grown overnight were diluted to 1:200 in fresh TSB medium, without antibiotics but supplemented with appropriate inducers (CdCl$_2$ at 0.1 μM for ColFtsZ$^{55–56}$sGFP, IPTG at 0.5 mM for ColWgZm and ColJgZm). After being collected, cells were re-suspended in M9 microsopy medium (KH$_2$PO$_4$ 3.4 g l$^{-1}$, K$_2$HPO$_4$ 2.9 g l$^{-1}$, VWR; di-ammonium citrate 0.7 g l$^{-1}$, Sigma–Aldrich; sodium acetate 0.26 g l$^{-1}$, Merck; glucose 1%, Merck; MgSO$_4$ 0.7 mg l$^{-1}$, Sigma–Aldrich; CaCl$_2$ 7 mg l$^{-1}$, Sigma–Aldrich; casamino acids 1%, Difco; 1× MEM amino acids, Thermo Fisher Scientific; MEM vitamins 1×, Thermo Fisher Scientific). The medium was supplemented when required with IPTG 0.5 mM, CdCl$_2$ 0.1 μM, DMPI 8 μg ml$^{-1}$ or PC190723 5 μg ml$^{-1}$. Cultures were spotted on a pad of 1.5% agarose in the same supplemented medium, and mounted in a gene on a microscope slide. The time between the cells contacting the pad and the start of acquisition was 5 min in all conditions. HADA incorporation microscopy assay. To assess whether Murf activity was required for HADA incorporation, the strains COL and ColDltC-GFP, which expresses a cytoplasmic DltC–sGFP fusion and can therefore be easily distinguished from COL under the microscope, were grown to an OD$_{600nm}$ of 0.4. At this point, each culture was separated into two flask and either DMPI (3 mg l$^{-1}$ in DMSO) or DMSO (0.2% final concentration) was added to the cultures. After 25 min of incubation, HADA (500 μM) was added to each culture for 5 min. Cells were then collected, washed twice with PBS (supplemented with DMPI when applicable) and DMSO-treated COL cells were mixed with DMPI-treated ColDltC-sGFP cells. To exclude the possibility that the expression of DltC–sGFP affected the results, a reverse experiment was performed in which cells of DMSO-treated ColDltC-sGFP were mixed with DMPI-treated COL. These mixtures of two strains were then imaged by fluorescence microscopy as described earlier.

Functionality test for EzrA–GFP construct. The strains COL and COL.EzrA were grown overnight in TSB, and the strain ColpSGEzrA-GFP was grown in TSB plus erythromycin 10 μg ml$^{-1}$, at 37°C with aeration. Cultures grown overnight were diluted to 1:200 in fresh TSB and incubated at 37°C with aeration. Once the OD$_{600nm}$ reached approximately 0.6, cells were pelleted, resuspended in PBS and spotted on a pad of 1.5% agarose in PBS. Cultures were imaged by phase contrast, then the single cells were identified and cell area measured (see below; a lack of EzrA produces larger colonies$^{40}$).

Imaging of FtsZ$^{55–56}$sGFP treadmilling by SIM. To visualize FtsZ$^{55–56}$sGFP movement in the strain ColFtsZ$^{55–56}$sGFP, and EzrA–GFP movement in the strain ColpSGEzrA-GFP, 50 frames of SIM images were acquired with 5-s intervals (total time of acquisition of 250 s) using the 488-nm laser at 50% and a 50-ms exposure time. For FtsZ, after this acquisition, 3 time-lapse images were taken 15 min apart to check whether the corresponding rings were constricting. These experiments were performed with or without PC190723 at 5 μg ml$^{-1}$. To analyse the movement of EzrA–GFP treadmilling, the 50 μm long 1-pixel lines were drawn over the rings and plotted in a kymograph.

Visualization of divisome ring constriction by SIM. To follow the constriction of FtsW–sGFP and Murf–sGFP rings in the strains ColFtsW–sGFP and ColMurf–sGFP, respectively, cultures were spotted on an agarose pad and visualized by wide-field microscopy. Assays were done in triplicate.

Label $S. aureus$ membranes were incubated with Nile Red (Invitrogen) at a final concentration of 10 μg ml$^{-1}$ for 5 min at room temperature, washed with PBS and then mounted on microscope slides for time-lapse experiments, all cultures were grown overnight in TSB.

For antisense RNA experiments, strains were grown until they reached an OD$_{600nm}$ of 0.1–0.2, at which point expression of the antisense RNA fragments was induced with xylose (Apollo Scientific) at a final concentration of 4% for 1 h. Cells were then collected and washed with PBS to remove xylose, mounted on microscope slides as described earlier and imaged by wide-field fluorescence microscopy. Assays were done in triplicate.

To evaluate localization of peptidoglycan synthesis activity, $S. aureus$ cells were given a pulse of HADA$^{17}$ (250 μM) for 1 min and then washed two times with PBS. Cells were then placed on an agarose pad and visualized by wide-field microscopy. Assays were done in triplicate.

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M9 pad with or without PC190723, and then imaged by SIM. The 488-nm laser was used at 100% intensity, with an exposure time of 50- ms. To decrease photodamage caused by high laser power, images were acquired every 10 min, instead of 5 every min, for 1 h. These settings were also applied to ColFtsZ55–56/sGFP for comparison. For MurJ–sGFP time-lapse images were also taken at 5-min intervals to confirm the absence of biphasic behaviour of the constriction ring. Only cells that did not show a MurJ–sGFP signal in the first frame but did present this signal in the second frame were analysed, to ensure that the entire constriction process was observed (that is, to confirm that the absence of a biphasic behaviour was not the result of only imaging cells in the later stages of cell division). After SIM image reconstruction, cropped stacks containing individual cells were used to plot constriction kymographs as described earlier.

Time-lapse stacks of ColFtsZ55–56/sGFP, ColFsTsW–sGFP and ColMurJ–sGFP in the presence of PC190723 were used to count the number of cells that had fusion at the midcell in the first frame and to determine the percentage of those cells that constricted, constricted with defects or did not constrict in the presence of PC190723 for 60 min. Data were plotted using GraphPad Prism 6.

To observe the constrictions of FtsW–sGFP and MurJ–sGFP rings in cells expressing FtsZ–mCherry, the strains ColWgZm and ColgGzm in M9 medium supplemented with IPTG were imaged by SIM every 10 min for 1 h, using the 488-nm laser and the 561-nm laser (100% laser power, 50- ms and 50% laser power, 50- ms, respectively). Cells of ColWgZm in which FtsZ–mCherry showed the brightest region inside the cell. This region is then defined as the septum. To separate the septum from the cytoplasm, the software uses the isodata algorithm to find the centres of each fluorescence channel were aligned and loaded side-by-side in eHooke. To measure the PCC between two fluorescent proteins in a strain, images of each fluorescence channel were aligned and loaded side-by-side in eHooke. After automatic cell segmentation, cells that showed a FtsZ signal at the septum were selected for PCC measurements. The pixels corresponding to each cell were isolated and PCC values between channels were then calculated using an equation adapted from ref. 43:

\[
PCC = \frac{\sum_i (X_i - \bar{X})(Y_i - \bar{Y})}{\sqrt{\sum_i (X_i - \bar{X})^2} \sqrt{\sum_i (Y_i - \bar{Y})^2}}
\]

in which \(X\) and \(Y\) correspond to each pixel intensity for two fluorescence channels \(X\) and \(Y\) correspond to the mean intensities of those channels.

**Statistical analysis.** Statistical analyses were done using GraphPad Prism 6 (GraphPad Software). Unpaired Student's t-tests were used to evaluate the differences of cellular volumes, and to compare fluorescence ratios between peripheral and septal wall signal intensities. Mann–Whitney U tests were used to compare differences between PCC non-normal distributions obtained in co-localization experiments. P values ≤ 0.05 were considered as significant for all analysis performed and are indicated with asterisks: *P ≤ 0.05, **P ≤ 0.01 and ***P < 0.001.

**Code availability.** Code is available from: https://github.com/BacterialCellBiologyLab/Bruno-Saraiva-2017.

**Data availability.** Source data for Figs 1–3 and 5 and Extended Data Figs 1–4, 7 and 9 are provided with the paper. All other data are available from the corresponding author upon reasonable request.

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Extended Data Figure 1 | Switching fluorescent tags has no effect on protein co-localization data. a, b, COL strains that express FtsZ–mCherry and FtsW–sGFP (a) or FtsZ–mCherry and MurJ–sGFP (b) were compared to strains that express FtsZ–CFP and FtsW–mCherry, and FtsZ–CFP and MurJ–mCherry, respectively (described in Fig. 2b, c). Scale bars, 2 μm. c, PCC values between fluorescence channels for each protein fusion pair were calculated for cells that showed septal FtsZ localization. From left to right, n = 138, 136, 133 and 139 cells. Negative PCC values are represented as 0. Data are represented as box-and-whisker plots in which boxes correspond to the first-to-third quartiles, lines inside the boxes indicate the median, and the ends of whiskers and outliers follow a Tukey representation. Statistical analysis was performed using a two-sided Mann–Whitney U test; ns, not significant. Images in a and b are representative of three biological replicates.
Extended Data Figure 2  |  Antisense RNA fragments that target the DivIB–DivIC–FtsL complex increased cell volume and decreased protein expression. **a**, Western blot that shows total protein extracts of ColJzpAS-DivIB after 1 h of antisense induction, and control ColJzpEPSA probed with antibodies against either PBP2A (loading control; upper bands) or DivIB (lower bands). **b**, Western blot that shows total protein extracts of ColJzpAS-DivIC after 1 h of antisense induction, and control ColJzpEPSA probed with antibodies against either PBP2A (loading control; upper bands) or DivIC (lower bands). Images in **a** and **b** are representative of three biological replicates. For gel source data, see Supplementary Fig. 1. **c**, Cell volume of cells that expressed antisense RNA against ftsL, divIB or divIC, or carried vector pEPSA (left to right, n = 421, 379, 279 and 361 cells). Data represented in column graphs in which the height of the column represents the mean and the whiskers are the s.d. Statistical analysis was performed using a two-sided unpaired t-test. ***P < 0.001; first versus second column, P = 3.50 × 10^{-6}; first versus third column, P = 3.80 × 10^{-8}; first versus fourth column, P = 1.27 × 10^{-29}. © 2018 Macmillan Publishers Limited, part of Springer Nature. All rights reserved.
Extended Data Figure 3 | FtsW and PBP1 recruitment to the divisome is independent of DivIB–DivIC–FtsL complexes. The strain ColWZpAS-FtsL, which harboured the FtsZ–CFP and FtsW–mCherry fluorescent fusion pair, and the strain ColP1ZpAS-FtsL, which harboured the FtsZ–mCherry and sGFP–PBP1 fluorescent fusion pair, were depleted of FtsL expression using antisense RNA and imaged by wide field fluorescence microscopy. a, Frequency of FtsZ–CFP and FtsW–mCherry co-occurrence in ColWZpAS-FtsL as compared to control ColWZpEPSA (n = 200 for each). b, Frequency of FtsZ–mCherry and sGFP–PBP1 co-occurrence in ColP1ZpAS-FtsL and in control ColP1ZpEPSA (n = 200 for each). c, d, Very large FtsL-depleted cells were observed either where FtsW–mCherry (c) or where sGFP–PBP1 (d) co-localized with the FtsZ fusion at the septum (arrows). e, f, Inhibition of divisome assembly at an early stage by depletion of FtsA expression in either ColWZpAS-FtsA or ColP1ZpAS-FtsA prevented the recruitment of FtsW–mCherry (e) or sGFP–PBP1 (f) to the septum, concomitant with FtsZ destabilization. Images in c–f are representative of three biological replicates. Scale bars, 1 μm.
Extended Data Figure 4 | Inhibition of MurJ activity does not prevent its recruitment to the midcell but impairs PG synthesis. a, Fluorescence microscopy images of ColMurJ-mCherry cells grown in the presence (left) or absence (right) of the MurJ inhibitor DMPI for 30 min at 2× minimum inhibitory concentration. Scale bar, 2 μm. b, c, Fluorescence microscopy images showing mixed cultures of either DMPI-treated ColDltC-sGFPi cells mixed with COL cells (b) or DMPI-treated COL cells mixed with ColDltC-sGFPi cells (c), after incubation with HADA. The two cultures, which can be easily distinguished owing to GFP expression in ColDltC-sGFPi, were mixed on the same slide to decrease the fluorescence variation of HADA signal that could result from imaging conditions. Data show that HADA incorporation (that is, PG synthesis) is greatly reduced in the presence of DMPI. Phase-contrast–GFP channel overlays are shown on the left, and phase-contrast–HADA channel overlays are shown on the right. Scale bars, 1 μm. d, e, HADA fluorescence signal measured at the midcell (midcell), the periphery (peripheral) or over the entire cell (total) of DMPI-treated ColDltC-sGFPi cells mixed with COL cells (d) or DMPI-treated COL cells mixed with ColDltC-sGFPi cells (e). Images in a–c are representative of three biological replicates. Data in d and e are represented as column plots (n = 100 cells for each column) in which the height of the column is the mean and the whiskers indicate s.d. Statistical analysis was performed using two-sided unpaired t-tests. ***P < 0.001. d, P = 2.34 × 10^{-38} for the midcell; P = 1.81 × 10^{-28} for the periphery; P = 9.22 × 10^{-34} for the entire cell. e, P = 1.74 × 10^{-33} for the midcell; P = 8.77 × 10^{-25} for the periphery; P = 7.60 × 10^{-26} for the entire cell.
Extended Data Figure 5 | Effect of antibiotics on cell-cycle progression of *S. aureus*. SIM images of Nile-Red-stained COL cells treated with DMPI, PC190723, oxacillin (Oxa), DMSO or TSB (mock-treated controls) for the duration of one cell cycle (30 min). Images are representative of three biological replicates. Scale bars, 1 μm.
Extended Data Figure 6 | Kymographs that show the constriction of FtsZ<sup>55–56</sup>sGFP and MurJ–sGFP rings.  

**a**. Kymographs of 10 cells per column that show the constriction of FtsZ<sup>55–56</sup>sGFP rings, obtained by imaging ColFtsZ<sup>55–56</sup>sGFP cells every 5 min for a total of 60 min (laser power 50%), in the absence (control) or presence of either PC190723 (+PC) or DMPI (+DMPI). Because *S. aureus* cells are not synchronised, cells at all stages of cytokinesis can be observed. Larger FtsZ<sup>55–56</sup>sGFP rings had a biphasic behaviour (no or slow constriction, followed by fast constriction) whereas smaller rings that are further ahead in the cell cycle were only observed undergoing the fast constriction step. The addition of PC190723 inhibited the constriction of larger rings (top two kymographs) but not of smaller rings, which were able to complete cytokinesis. The addition of DMPI completely blocked constriction of FtsZ<sup>55–56</sup>sGFP rings of all sizes. 

**b**. Kymographs showing constriction of MurJ–sGFP rings in ColMurJ-sGFP cells imaged every 5 min for a total of 60 min (laser power 100%). Cells in which a MurJ–sGFP signal appeared on the second frame were chosen for analysis to ensure that the entire constriction process was imaged. Fast constriction started immediately on the arrival of MurJ–sGFP at the division septum and therefore rings did not show biphasic behaviour. Data are representative of three biological replicates. Scale bars, 0.5 μm.
Extended Data Figure 7 | The Z ring protein EzrA shows impaired treadmilling in the presence of PC190723 and biphasic ring constriction. a, ColpSGEzrA-GFP cells that express a functional EzrA fusion to GFP were imaged by SIM every 5 s in the absence (EzrA control) or presence (EzrA + PC) of PC190723. Kymographs were obtained by extracting fluorescence intensity values along the red line indicated in cells in the left panels. Similar to the observations for FtsZS55–56sGFP, the addition of PC190723 abolished EzrA movement (vertical lines in the kymographs).

b, ColpSGEzrA-GFP cells were imaged by SIM every 5 min in the absence (left) or presence (right) of PC190723, and kymographs that show the constriction of EzrA–GFP rings were plotted. Under control conditions, the larger EzrA rings showed biphasic constriction behaviour whereas in the presence of PC190723 only rings in the second stage of cytokinesis were able to constrict. Data in a and b are representative of two biological replicates. Scale bars, 1 μm.

c, To test the functionality of the EzrA–GFP construct, the strains COL, ColpSGEzrA-GFP and COLΔEzrA (which lacks ezrA) were imaged by phase contrast and cell areas were measured. The lack of EzrA in COLΔEzrA (n = 959) resulted in cell enlargement, whereas the size distribution of ColpSGEzrA-GFP (n = 957) cells mimicked that of parental strain COL (n = 851), which indicates that the EzrA fluorescent fusion is functional.
Extended Data Figure 8 | Kymographs that show the constriction of FtsZ55–56sGFP, FtsW–sGFP and MurJ–sGFP rings during cell division. The strains ColFtsZ55–56sGFP, ColFtsW-sGFP and ColMurJ-sGFP were imaged every 10 min in the absence (control) or presence (+PC) of PC190723 for a total of 60 min. MurJ–sGFP control kymographs were performed on cells in which the MurJ–sGFP signal appeared on the second frame to ensure that the entire constriction process was observed (that is, to confirm that the absence of a biphasic behaviour was not the result of only imaging cells in the later stages of cell division). FtsZ55–56sGFP and FtsW–sGFP rings showed biphasic constriction behaviour (no or slow constriction, followed by fast constriction). The addition of PC190723 inhibited the constriction of larger FtsZ55–56sGFP and FtsW–sGFP rings (top kymographs) but not of smaller rings that were undergoing fast constriction. MurJ–sGFP rings displayed only fast constriction, and therefore were always able to constrict in the presence of PC190723. Data are representative of three biological replicates. Scale bars, 0.5 μm.
Extended Data Figure 9 | Graphs of FtsZ, MurJ and FtsW ring diameter during constriction. a, Graphs of ring diameters of FtsZ–mCherry (red) and MurJ–sGFP (blue) in strain ColJgZm. SIM images were taken every 10 min and measurements of ring diameter of FtsZ–mCherry and MurJ–sGFP were performed in cells in which a MurJ–sGFP signal first appeared after 20 min. b, Graphs of ring diameters of FtsZ–mCherry (red) and FtsW–sGFP (blue) in the strain ColWgZm. SIM images were taken every 10 min and measurements of ring diameter of FtsZ–mCherry and FtsW–sGFP were performed in cells in which FtsZ–mCherry ring diameter was constant for at least the first 20 min.
Extended Data Figure 10 | Model for cytokinesis in *S. aureus*. a, Cells in phase 1 of the cell cycle (before septum synthesis is initiated) synthesize peptidoglycan at the cell periphery. b, In preparation for division, FtsZ and early components of the divisome assemble at the midcell. c, At this stage, cells initiate the first, slow step of cytokinesis (which is dependent on FtsZ treadmilling) and FtsZ may act as the driving force for the initial invagination of the membrane. d, MurJ then arrives at the divisome, in a process dependent on the presence of the sub-complex DivIB–DivIC–FtsL, bringing the PG precursor (lipid II) flippase activity to the midcell. The major *S. aureus* PG synthase (PBP2) is recruited to the midcell through substrate (translocated lipid II) recognition, and massive PG synthesis is initiated to synthesize the septum. From this point on, cytokinesis no longer depends on FtsZ treadmilling and is most probably driven by PG synthesis.
Experimental design

1. Sample size
   Describe how sample size was determined.
   No sample size calculation was made. In bacterial microscopy it is usual to quantify data from at least 100 cells. Our minimal sample size was 136, but for most experiments n>300.

2. Data exclusions
   Describe any data exclusions.
   No data was excluded from the analysis, except for timelapses in Fig 6a and c in which cells that did not divide for 60 min were excluded from the analysis.

3. Replication
   Describe whether the experimental findings were reliably reproduced.
   All replicates done in identical conditions were successful except, as mentioned above, for timelapses in Fig 6a and c, where occasionally cells did not divide during the 60 min of the experiment.

4. Randomization
   Describe how samples/organisms/participants were allocated into experimental groups.
   Samples were not allocated to groups

5. Blinding
   Describe whether the investigators were blinded to group allocation during data collection and/or analysis.
   Blinding was not relevant as samples were not allocated to groups

Note: all studies involving animals and/or human research participants must disclose whether blinding and randomization were used.

6. Statistical parameters
   For all figures and tables that use statistical methods, confirm that the following items are present in relevant figure legends (or in the Methods section if additional space is needed).

   n/a  Confirmed
   □   □ The exact sample size (n) for each experimental group/condition, given as a discrete number and unit of measurement (animals, litters, cultures, etc.)
   □   □ A description of how samples were collected, noting whether measurements were taken from distinct samples or whether the same sample was measured repeatedly
   □   □ A statement indicating how many times each experiment was replicated
   □   □ The statistical test(s) used and whether they are one- or two-sided (note: only common tests should be described solely by name; more complex techniques should be described in the Methods section)
   □   □ A description of any assumptions or corrections, such as an adjustment for multiple comparisons
   □   □ The test results (e.g. P values) given as exact values whenever possible and with confidence intervals noted
   □   □ A clear description of statistics including central tendency (e.g. median, mean) and variation (e.g. standard deviation, interquartile range)
   □   □ Clearly defined error bars

See the web collection on statistics for biologists for further resources and guidance.
Software

7. Software
Describe the software used to analyze the data in this study.

- SIM microscopy acquisition and reconstruction was done using ZEN software (black edition, 2012, version 8.1.0.484).
- Epifluorescence images were acquired using Metamorph 7.7.0.0 or Zen (blue edition, version 2.0.0.0).
- Image analysis was done using Fiji vs 1.49.
- Statistical analysis was made using GraphPad Prism 6.
- Data in Figs 1c, 2c, 3a, and 5, as well as extended figure 1c was obtained using custom software which is deposited in GitHub and link is given in manuscript.

For manuscripts utilizing custom algorithms or software that are central to the paper but not yet described in the published literature, software must be made available to editors and reviewers upon request. We strongly encourage code deposition in a community repository (e.g. GitHub). Nature Methods guidance for providing algorithms and software for publication provides further information on this topic.

Materials and reagents

8. Materials availability
Indicate whether there are restrictions on availability of unique materials or if these materials are only available for distribution by a for-profit company.

- The FDAAs are available upon request from mvannieu@indiana.edu

9. Antibodies
Describe the antibodies used and how they were validated for use in the system under study (i.e. assay and species).

- Antibodies against DivIB and DivIC were obtained from Simon Foster, University of Sheffield.
  - DivIB antibody was validated using a mutant where divIB gene expression was placed under the control of an inducible promoter inducible. The band recognized by the DivIB antibody disappears in the absence of the inducer (which leads to DivIB depletion). Data published in Fig. 5C in doi: 10.1111/mmi.12813.
  - DivIC antibody was validated by performing a Western blot against pure DivIC protein (data available in http://etheses.whiterose.ac.uk/6487/1/Thesis%20corrected%20version-Azhar.pdf, Fig. 4.1)

10. Eukaryotic cell lines
a. State the source of each eukaryotic cell line used.
  - No eukaryotic cell lines were used

b. Describe the method of cell line authentication used.
  - No eukaryotic cell lines were used

c. Report whether the cell lines were tested for mycoplasma contamination.
  - No eukaryotic cell lines were used

d. If any of the cell lines used are listed in the database of commonly misidentified cell lines maintained by ICLAC, provide a scientific rationale for their use.
  - No eukaryotic cell lines were used

Animals and human research participants

11. Description of research animals
Provide details on animals and/or animal-derived materials used in the study.

- No animals were used

Policy information about studies involving human research participants

12. Description of human research participants
Describe the covariate-relevant population characteristics of the human research participants.

- No human participants were involved