Description of Additional Supplementary Files:

Supplementary Video 1: Aggregates perfusability in the bacterial confiner. Timelapse confocal images acquired at 1 minute frame rate of aggregate perfusability in the bacterial confiner using 10 kDa Dextran-FITC in absence of confinement (blue) and presence of confinement (3.5 h after pressure build-up, orange). Time 0 corresponds to the time 1 min before Dextran injection. Time is indicated as hh:mm. Scale bar: 5 μm. See also Supp. Figure 1.C-D.

Supplementary Video 2: Bacterial proliferation under confinement generates growth-induced pressure (GIP). Timelapse brightfield images acquired at 30 minutes frame rate and segmented chamber edges of bacterial proliferation in the bacterial confiner in the absence of confinement (blue, left), and presence of confinement (orange, right). The frame corresponding to the onset of growth-induced pressure (GIP) is highlighted. Time is indicated as hh:mm. Scale bar: 5 μm. See also Figure 1.B-C and Supp. Figure 1.F-I.

Supplementary Video 3: Growth-induced pressure leads to rod cell shortening due to uncoupling between bacterial growth and division. Timelapse confocal images acquired at 30 minutes frame rate of *E. coli* MG1655 ZipATM-mCherry bacteria fluorescently labeled at the inner membrane in the absence of confinement (blue, left), and presence of confinement (orange, right), and corresponding colormap of the bacterial area after single-cell segmentation. Finally, representative inset of a sequence of reductive divisions induced by pressure build-up in the chamber. The frame corresponding to the onset of growthinduced pressure (GIP) is highlighted. Time is indicated as hh:mm. Scale bar: 5 μm. See also Figure 2.A.

Supplementary Video 4: Cytoplasmic crowding increases during early confinement and is sufficient to explain growth and division uncoupling. In a sequence, timelapse confocal images of *E. coli* MG1655 GFP ZipATM-mCherry bacteria expressing in their cytoplasm a constitutive GFP (to note, no autoscale/frame was applied in the corresponding Movie), *E. coli* HU-GFP ZipATM-mCherry bacteria fluorescently labeled at the inner membrane and DNA and *E. coli* 40NM-GEMS ZipATMmCherry bacteria expressing diffusive nanoparticles in their cytoplasm during proliferation in the bacterial confiner. When required, conditions in the absence of confinement (blue) and presence of confinement (orange) are shown. The frame corresponding to the onset of growthinduced pressure (GIP) is highlighted. Time is indicated as hh:mm. Scale bar: 5 μm. See also Figure 3.A, D, and F.

Supplementary Video 5: Transcriptional responses upon mechanical confinement. In a sequence, timelapse confocal images acquired at 30 minutes frame rate of the *E. coli* MG1655 ZipATM-mCherry strain expressing PrcsA-GFP, PcpxP-meGFP, and PrecA-YFP during proliferation in the bacterial confiner in the absence and in the presence of confinement (top left and bottom left, respectively). The frame corresponding to the onset of growthinduced pressure (GIP) is

highlighted. Time is indicated as hh:mm. Scale bar: 5 µm. See also Figure 5.A, Supp. Figure 6.B, E, and H.

Supplementary Video 6: Morphology of cell envelope mutants upon mechanical confinement. In a sequence, timelapse confocal images acquired at 30 minutes frame rate of the *E. coli* MG1655 ZipATMmCherry mutant strains in rcsB, cpxR and wcaJ during proliferation in the bacterial confiner. Conditions in the absence of confinement (blue, left) and presence of confinement (orange, right) are shown. The frame corresponding to the onset of growthinduced pressure (GIP) is highlighted. Time is indicated as hh:mm. Scale bar: 5 µm. See also Figure 5.C, Supp. Figure 6.L-M.

Supplementary Video 7: Confinement induces similar morphological changes in a pathogenic strain that are reversible upon pressure release. Timelapse confocal images acquired at 30 minutes frame rate of pathogenic *E. coli* UTI89 RFP bacteria before, during confinement and after pressure release. The frame corresponding to the onset of growth-induced pressure (GIP) is highlighted. Time is indicated as hh:mm. Scale bar: 5 µm. See also Supp. Figure 7.A and Supp. Figure 3.G-H.