

Figure S1. Growing sites of Zm6 cells visualized by a HADA-labeling. Zm6 cells growing under anaerobic conditions were stained with HADA at 500 µM for 30 minutes under a dark condition. The cells were then washed with PBS twice and mounted on PBS agarose pad and imaged by a fluorescence microscope. The labeling showed that Z. mobilis cell incorporated new cell wall materials at its division site and lateral walls. Left panel; a phase contrast image. Middle; an overlay of phase contrast and fluorescence images. Right; a fluorescence image. Blue; HADA. Scale bar; 4 µm.



16 Figure S2. Histogram and Kernel density plot of cell length in growing Z. mobilis strains Z6KF1 and Z6KF2. Growing Z6KF1 (OD₆₀₀ 0.8) and Z6KF2 (OD₆₀₀ 0.32) 17 culture was directly mounted on PBS agarose pad and imaged by phase contrast 18 19 microscopy. The mean of cell length was 2.78 μ m for Z6KF1 (*n* = 79) and 2.83 μ m for Z6KF2 (n = 104). The bandwidth used for the Kernel density plot was 0.299 for 20 Z6KF1 and 0.272 for Z6KF2. To test the functionality of FtsZ-GFP fusion protein, we 21 first examined if the expression altered morphology or cell size in the strain Z6KF2. 22 Despite that overexpression of *ftsZ* is known to cause reduction of cell size in several 23 24 bacteria, the cells did not show any obvious morphological changes. The result suggested two possibilities; an overexpression of functional FtsZ-GFP did not alter 25 cell function in the strain, or that the fusion protein was not functional and did not 26 27 cause any dominant negative effects. The strain Z6KF1 carrying intact ftsZ in the pBBR plasmid, exhibited no obvious morphological defects either, indicating that 28 overproduction of functional FtsZ by the pBBR1 plasmid was not overwhelming the 29 native production of Zm6 FtsZ. Note that same upstream promoter was used for 30 expressions of *ftsZ* in Z6KF1 and *ftsZ-GFP* in Z6KF2. These phenotypic 31 observations, together with subcellular localization of the Z. mobilis FtsZ-GFP fusion 32

- in the strain Z6KF2, indicated that FtsZ-GFP did not cause much disturbance to the
- 34 cellular organization in the expressed strain.

35			
36			
37			
38			
39			
40			
41			
42			
43			
44			
45			
46			





Figure S3. Box and whisker plot of ratio of short:long cell compartment length in growing *Z. mobilis* strains Z6KF2. Cell compartment length was defined as distance between a cell pole to Z-ring position. The box shows the ratio of short:long cellcompartments. The box is marked with mean (x) and median (middle line of the box). Note that the mean value of the cell compartment ratio (0.833) is very close to the ratio of dividing daughter cells (0.837) in the Figure 1, showing that Z-ring positions represent division sites in *Z. mobilis* cells. *n* = 106.

56

57

58



Figure S4. Plot of normalized FtsZ-GFP peak intensity within a cell by a function of its cell length. The linear relationship indicates that cells accumulated FtsZ ring linearly along with cell growth. Perason's r = 0.8, p-value < 0.001. n = 80.







75	Figure S5. FtsZ-GFP sub-cellular localizations in salt stress-induced filamentous
76	Z6KF2 cells. The Z6KF2 culture was grown in the complex medium supplemented
77	with 0.2 M NaCI. The growing filamented cells were directly mounted on agarose pad
78	of the identical growth medium, and fluorescence imaging was performed. FtsZ-GFP
79	formed either foci or filamentous structured in the elongated cells. Left; phase
80	contrast image. Right; fluorescence image. Green; FtsZ-GFP. Scale bar; 5 μ m.
81	
82	
83	
84	
85	
86	
87	
88	



Figure S6. Visualizing Z. mobilis nucleoids by different staining methods. The staining 90 91 was to examine if variant FL signal intensities found in Zm6 cells (Fig. 5A, 5C) were due to possible artifacts from the staining procedure. (A) Growing Zm6 cells stained 92 with DAPI 50 µg/ml for 20 minutes. (B) Growing Zm6 cells stained with SYTO 9 at 11 93 µM for 10 minutes. (C) SYTO 9 staining of chemically fixed Zm6 cell. The cells were 94 fixed by 10% formalin (v/v) for half hour, washed with PBS and stained with SYTO 9 95 at 0.5 µM. The cells were washed with PBS in all tested methods before imaging. All 96 three staining procedures resulted in the variant FL signal intensities between Zm6 97 cells. The result of (A) ruled out a possibility of dye-specific artefact as a cause of the 98 variant FL signals among individual Zm6 cells. The result of (B) excluded a possibility 99 of artefact caused by a depletion of dye in (Fig. 5A). The result of (C) excluded a 100 possibility of stress-response based phenomenon to cause the variant FL intensities 101 102 in (Fig. 5A). White; fluorescence signals from the dyes. Scale bar; 10 µm.



Figure S7. Plot of cell DNA crowdedness ratio by a function of ratio of cell length between paired-sibling cells (short:long cell length). DNA crowdedness/density was an average of FL signal intensities within cell. Spearman's rank correlation test; Rs = 0.3, p-value > 0.001, *n* = 90.



Figure S8. Inclined Z-rings in Z6KF2 cells. (A) Short time-lapse imaging of Z6KF2 114 cells anaerobically growing on the complex medium agarose pad. Left panels, 115 overlays of phase contrast and fluorescence images (green: FtsZ-GFP). Right 116 panels, phase contrast images. Numbers indicate minutes after imaging started. (B) 117 Single time imaging of growing Z6KF2 cells. Images are overlays of phase contrast 118 and fluorescence images. Note that Z-rings were leaned from the short axis in some 119 of growing Z6KF2 cells. White arrowheads indicate inclined Z-ring in all images. 120 Scale bars; 2 µm for all images. 121



- 123 Figure S9. Electron micrographs of the strain Zm6 cell (left) and the strain Zm4 cells
- 124 (right). Arrowheads indicate constriction sites that are slightly inclined from short
- vertical axis of cells.